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Effects of Short Term Dietary Nitrate Supplementation on Energy Metabolism during Isokinetic Knee Muscle Contractions

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Effects of Short Term Dietary Nitrate Supplementation on Energy Metabolism during Isokinetic Knee Muscle Contractions

by

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Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

University of Texas at Austin

December 2014

Acknowledgements

I want to mention those who supported me to complete my thesis project. First of all, I would like to thank my academic advisor, John L. Ivy, for giving me great opportunity to study in the Exercise Physiology and Metabolism Laboratory and to investigate how nitrate supplementation affects energy metabolism during resistance exercise. Robert M. Brothers also should be mentioned for his support from preparing Institutional Review Board (IRB) documents to reviewing thesis writing as a thesis supervisor. I think I could not finish my thesis study without the cooperation of lab colleagues. They contributed to make each experimental trial consistent throughout the study although the daily study schedule was pretty tight. Zhenping Ding gave me his know-how about details of the blood sample handling and assay with his skillful experience and participated in randomization of subjects. As a main Tech 2, Geffory Solares handled the blood sample aliquots and storage and also contribute to data organization and analysis. As a main Tech 1, Bo Wang took care of metabolic cart handling, specific time recording, and measurement of blood pressure and heart rate. Brian Lisco and Dongwoo Hahn as pilot study subjects, Sheng-Ju Chung and Hans Kristian Stadheim as a Tech 1, Kiyoung Kim as a Tech 2, and Wayni Wang and Soon Mi Choi with phlebotomy advice deserve my special thanks. Brian Farr as a Department Review Committee (DRC) chair, Elizabeth Husley as an administrative associate, Philip Salazar as a graduate program coordinator, and Philip Stanforth as a FIT Director also gave me their special supports during research duration.

Lastly, I want to give my special thanks to my wife, Jihee Suh and our families who love and encourage me to keep studying with their trust and support.

Abstract

Effects of Short Term Dietary Nitrate Supplementation on Energy Metabolism during Isokinetic Knee Muscle Contractions

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The purpose of this study was to investigate whether 3 days of dietary nitrate supplementation has positive effects on exercise performance and energy metabolism at rest and during strenuous knee exercise, and exercise recovery. The experimental protocol was a double blind, placebo-controlled, two-period, and within-subjects design. Fourteen healthy nonsmoking males (22.71 ± 0.72yr, 72.93 ± 2.35kg, and 47.67 ± 1.56 ml/kg/min VO_{2max}) participated in the study. Two supplements (nitrate lozenge; NO-L or placebo; PLA) were orally administrated randomly at 48 and 24 hours before each trial day and again 40 minutes before each exercise trial. Total work, peak torque, and respiratory gases were automatically collected during repeated knee extensions/flexions (isokinetic concentric contractions with dominant leg; 4 sets of 28 repetitions at 180°/sec with 30 sec rest intervals) and recovery (6 contractions of 1 repetition maximum with 120

sec between contractions). For these results, peak torque, rate of fatigue, work efficiency, and rate of recovery were calculated. Blood specimens were collected at rest before and after the treatment, post exercise, and end of recovery to track the changes in blood glucose and lactate concentrations. There was a significant inverse correlation for total work during knee extension exercise and oxygen consumption (PLA: $r = -.560$ and NO-L: $r = -.546$; $p < .01$, respectively). During the exercise, RER was significantly higher for PLA compared with NO-L (PLA: 1.42 ± 0.02 vs. NO-L: 1.38 ± 0.02: $p = .03$). Work within each set and total work performed were higher for NO-L, but these differences were not significant. However, NO-L enhanced exercise efficiency by 3.3% when compared with PLA (PLA: 2497.5 ± 134.56 ft-lb/L/min vs. NO-L: 2578.7 ± 132.24 ft-lb/L/min; $p = .05$). Extensor peak torque recovery rate slope was not significant, but meaningfully faster for NO-L (PLA: $2.39 \pm 0.52\%$ vs. NO-L: $3.06 \pm 0.54\%$; $p = .09$) and significant interaction effect ($p = .02$) was found during recovery contractions, especially from contractions 2 to 3 ($p = .03$). It is also worth noting that differences in time to peak torque during knee extension exercise for NO-L and PLA approached significances (PLA: 0.213 ± 0.01 sec vs. NO-L: 0.200 ± 0.01 sec; $p = .08$). The results suggest that 3 days of dietary nitrate supplementation improves rate of exercise recovery and enhances work efficiency during vigorous resistance exercise.

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INTRODUCTION

For those who must prove their performance in competitive sport events, it is important to have a well-organized daily training program. It is also important for those who wish to challenge their physical limits. The nutritional supplement market has been developed and various ergogenic aids covering physical to psychological effectiveness have been introduced to the fields (Kreider et al., 2010).

Individual supplements affect exercise performance through supplement-specific mechanisms in the human body. For example, caffeine supplementation improves endurance performance by enhancing free fatty acid utilization (Ivy et al., 1979), while creatine monohydrate supplementation is mostly used in strength training with the purpose of promoting anaerobic capacity by directly increasing high phosphate energy stores (Jäger et al., 2011).

There is a rising supplement, inorganic dietary nitrate, which is a potent exogenous source of nitric oxide (NO) (McKnight et al., 1999). The key function of NO in the body is to relax smooth muscle and expand blood vessels and thus increase blood flow (Pearson & Vanhoutte, 1993). It is now well known that NO also increases the ATP to oxygen (P/O) ratio in the mitochondria by reducing the leakage of protons by reducing expression of uncoupling protein 3 (UCP3) and adenine nucleotide translocator (ANT) (Larsen et al., 2011A). In addition, Bailey et al. (2010) suggested NO reduced the ATP turnover rate during muscle contraction, thus increasing exercise efficiency during repeated knee muscle extension contractions. Based on the knowledge of prior studies, many exercise scientists have focused on dietary nitrate supplementation, which includes various types of treatments such as sodium nitrate, sodium nitrite, and beetroot juice (Bescós et al., 2012B). Studies continuously support the claim that augmenting NO bioavailability in the blood after the intake of nitrate supplements results in

positive effects on blood pressure (Vanhatalo et al., 2011), the oxygen cost of exercise (Cermak et al., 2012A), and the exercise performance (Wilkerson et al., 2012 & Fulford et al., 2013).

The Need for Research

Most of the knowledge established in support of dietary nitrate supplementation has been based on sub-maximal (Larsen et al., 2007 & Bailey et al., 2009) or endurance exercise (Wilkerson et al., 2012 & Cermak et al., 2012) and thus there is a lack of information on how dietary nitrate supplementation affects performance and metabolism during resistance exercise on high intensity short duration, intermittent maximal intensity exercise. Blood nitrate and nitrite levels reach peak values approximately 1.5 hours to 3 hours, respectively after acute nitrate supplementation (Webb et al., 2008 & Wylie et al., 2013A) and thus most studies that report positive effects were performed at the nitrite peak time, which means 2.5 to 3 hours after supplement consumption (Lansley et al., 2011 & Bescós et al., 2012). Short-term nitrate supplementation studies, on the other hand, have shown that 2 to 5 days administration prior to testing significantly increases the plasma nitrite level, but actual exercise tests were conducted at least 2 to 2.5 hour after last nitrate load. Considering the interval between supplementation and exercise, this approach is inconvenient for the athlete. Based on these limitations, a new nitrate lozenge (NO-L) that generates NO rapidly was investigated. NO-L contains a natural source of nitrate and nitrite that can be converted to NO in the oral cavity. In our previous study about acute NO supplementation, we found significant improvement in cycling time trial performance without a difference in oxidative phosphorylation efficiency. Since the additional NO effects on metabolic efficiency have been typically found after 3~6 days of nitrate administration, we took this into consideration when administering the NO-L.

Purpose

The purpose of this study was to determine whether 3 days of NO-L supplementation advances the peak blood NO level and timing with positive effects on exercise performance and energy metabolism during intense repeated isokinetic knee muscle contractions.

Hypotheses

We hypothesized that short-term NO-L supplementation would

1. Improve hemodynamic indicators throughout the trials
2. Increase total work or reduce oxygen cost and thus improve work efficiency
3. Decrease rate of fatigue and increase rate of leg force recovery

METHODS

Before recruiting subjects, the present study was approved by The Institutional Review Board (IRB) of the University of Texas at Austin and the study protocol was planned as a double blind, randomized, placebo-controlled, and within-subjects design (Appendix C1).

Subjects

As study subjects, fourteen healthy nonsmoking male volunteers between 20 and 28 years old were recruited from the Austin, Texas area. The 50^{th} percentile of VO_{2max} value (42.5ml/kg/min) based on sex and age was used as the criterion to screen out unfit subjects. During the period of the study (Below), each participant was required not to be involved in any other studies and not to intake any other supplements except NO-L and PLA.

Time Period of the Study

Experiment period for each subject was about 1 month and consist of 4 visits to the laboratory. From visit 2, each visit was spaced 1 week apart (Appendix C2).

Visit 1	Visit 2		Visit 3		Visit 4
(Screening)	(Familiarization)	1 week	(Trial one)	1 week	(Trial two)

Screening Test

After signing The Informed Consent Form (ICF) and completing the health screener, each subject's basic information of resting blood pressure, height, weight, and dominant leg was collected. Potential subjects then completed a VO_{2max} test on a computer-controlled cycle ergometer (Velotron, Racer Mate, USA). The protocol for VO_{2max}test (Appendix B1) consisted

of a warm-up followed by 1min stages of increasing difficulty until fatigue. The subject breathed expired gases into a mixing chamber for analysis of pulmonary ventilation (VE), oxygen consumption (VO_2), and carbon dioxide production (VCO_2). Inspired gases were collected and analyzed using a computer-controlled metabolic cart (Pravo Medics, True One 2400, USA). The criteria used to establish VO_{2max} were a plateau in VO_2 with increasing exercise intensity, respiratory exchange ratio (RER) > 1.10, rated perceived exertion (RPE) > 17 on the Borg scale, and pedaling of minimum revolutions per minute (RPM) < 60. Afterward the subjects were given a cool-down (5 min) period and rest interval (20~30 min) to recover from the VO_{2max} test prior to starting the knee exercise (isokinetic concentric contraction dominant leg only) practice. Proper explanation and feedback about the position and kicks were given during the practice. From this first visit, identical knee exercise protocol [(exercise protocol; 4 sets of 28 repetitions at 180°/sec angular velocity of 90° range of motion (ROM) with 30 seconds interval between sets) and (recovery protocol; 6 contractions of 1 repetition maximum at 180°/sec angular velocity of 90° ROM with 120 seconds interval between contractions)] was used in order to minimize the learning effect (Appendix B2).

Familiarization

Procedure for the familiarization was identical with the experimental trials (Appendix B3) except that subjects did not receive a supplement and no blood samples were drawn. During familiarization, all subjects were required to have a catheter inserted into their forearm vein so that the risk of an uncomfortable feeling and fainting should be minimized during the experimental trials. In addition, the investigator informed provided instruction on how to take the

study supplements (Appendix C4) and set the time of testing of each subject based on supplementation and blood sampling.

Experimental Trials

All subjects were required to consume a total three lozenges of NO-L or PLA two days before each trial (1 lozenge/day). A reminder email was sent 3 days before the trial to ensure compliance with supplementation guidelines, and provide general information and instructions. Two text messages were also followed 2 days and 1 day before the trial to monitor supplementation and to determine if subjects were complying with guidelines for daily activity.

Once subjects arrived at the lab, questions about the information of drinking caffeine or alcohol and mouth washing were asked and body mass was measured. Drinking water was inhibited until to the end of the study. To check the fasting status, blood glucose was measured by using a finger prick and glucose meter (Life Scan, OneTouch Ultra, USA). If the fasting blood glucose was less than 100 mg/dl, a heart rate monitor (Cardio Sport, CE0434, Taiwan) was placed on the center of the subject's chest. A catheter with extension tube was inserted into the subject's forearm vein and flushed with 0.9% saline every 6-7min throughout the testing. A 5 ml blood sample was collected (BD1) and proper centrifugation and aliquot were followed by blood handling method (Appendix B7). The first blood pressure (BP1) (Omron Healthcare Inc, Bannockburn, USA) reading was then taken 2~3 min after resting. To collect pre-treatment basal metabolic gases subjects on the Biodex were required to maintain a relaxed static position during the 5 min measurement (Appendix B4.1). After the measurement of pre-treatment resting respiratory gases, subjects consumed the 3rd lozenge and rested for 30 min without any activity.

At around 27 min, Subjects were positioned for the exercise protocol and 5 min post-treatment metabolic gases taken along with a second blood sample and a second blood pressure reading.

Isokinetic knee exercise was initiated at 40 min post supplementation and then a third blood sample and blood pressure were collected during the 2 min interval between 4th set and first recovery contraction. After the blood sample, 6 recovery contractions followed. During exercise (4 min) and recovery (12 min) phases, respiratory gas samples and heart rates were continuously collected and monitored (Appendix B4 and C5). To obtain the 5th blood sample, subjects were reported to stop by the laboratory approximately 24 hours after the exercise and twelve out of fourteen subjects met the sampling schedule.

Supplementation and Diet Control

In the present study, NO-L and PLA were provided by Neogenis Lab (Austin, TX). NO-L contains 420mg of nitrate and nitrite blend and PLA was matched NO-L as a similar color and flavored non-caloric. Both supplements were formed as a lozenge type, which were able to be dissolved in the oral cavity with saliva within three minutes. Subjects received study products in random order approximately 1 week apart. Supplementation was designed for subjects to consume a total of 3 lozenges among which the first lozenge was taken two days, and the second lozenge one day prior to each experimental trial. The last lozenge was taken 40 minutes before the exercise test commenced. Only a member of our lab who had no direct contact with the subjects controlled randomization. To avoid an antibacterial effect on nitrate reduction in the mouth, the subjects were required to avoid using mouthwash during the whole time involved in the study. For two days prior to each exercise trial, subjects were required to eat similar types of foods and amounts around the same time, and to record foods consumed and exercises performed

on myfitnesspal.com. However, the type of foods consumed were not controlled could have contained a high nitrate concentrations. From the familiarization to the second trial, every test began with a 12-hour food and caffeine fast and with 24-hour vigorous exercise and alcohol abstention.

Blood Handling

Blood samples (5ml each) were collected at rest pre-treatment (BD1) and post-treatment (BD2), post exercise (BD3), and post recovery (BD4). Approximately, 2ml of 5ml of blood was transferred to a 12 × 17 test tube and then 1ml for analysis of NOx was mixed with 0.05ml of EDTA (24 mg/ml, pH 7.4) and 0.4 ml for lactate was mixed with 0.8ml of 10% perchloric acid (PCA). These micro-tubes were centrifuged for 15 minutes at 1,700 RCF (Eppendorf AG, Centrifuge 5424, USA). 0.25 ml NOx plasma was immediately transferred into 0.25 ml of methanol, mixed, and additionally centrifuged for 15 minutes at 15,000 RPM (Appendix B5). Supernatants of the NOx and lactate were transferred into the pre-labeled empty micro-tubes (Appendix B6) and kept in ice (0°C) until every blood sample was collected. Approximately, 3ml blood was transferred directly into a 4.5ml test tube and centrifuged for 15 minutes at 1,700 g in a JS-7.5 rotor in a Beckman J2-21 centrifuge. After centrifugation, the serum was stored at -80°C and later used for determination of blood lactate, glucose, and creatine phosphate (Appendix B7.1).

Data Collection and Analysis

Total work, peak torque, fatigue rate, and recovery rate were used to evaluate effects of NO-L on knee exercise performance, and metabolic gases were collected and calculated to determine the oxygen cost and work efficiency. Blood lactate and glucose samples were

analyzed by using enzymatic methods. Blood NOx samples were sent to The University of Texas Health Science, Huston for analysis.

Isokinetic Performance Assessment

Since the data provides unidirectional total work for extension and flexion, to determine total work for each set, extension total work and flexion total work for each set were summed, and to get entire total work, every set total work was summed. Single peak torque was measured as the highest value of torque developed throughout the range of motion. Peak torque used in this study was determined by averaging the three highest peak torque values among the first six repetitions of each set. In the same way lowest torque was also averaged among the last six repetitions per set. Time to peak torque is a measure of time from the start of the knee muscle contraction to the point of highest torque development. This index can be used for functional ability to produce torque quickly. Fatigue rate was defined as a rate of decline of peak torque during exercise and was calculated by the slope of each set peak torque. Recovery rate was defined as a rate of incline of peak torque during recovery contractions and was calculated by the slope of each contraction peak torque.

Blood Tissue Analysis

Collected blood tissues were stored at -80°C until analysis. To attain consistent data, standard sample was used and every sample was duplicated.

Lactate

Blood lactate concentration was measured by the method of Hohorst (1963). LDH in the reagent cocktail convert Lactate and NAD into pyruvate and NADH. The reaction took place in a glycine – hydrazine buffer, which allowed pyruvate to interact with hydrazine and the reaction to run to completion of assay. The spectrophotometer (Beckman, DU640 spectrophotometer) was set a 340 nm wavelength for detection of NADH. The amount of NADH produced was directly related to the amount of lactate.

Glucose

Glucose oxidase reagent kit (POINTE SCIENTIFIC, INC) contains glucose oxidase >15U/ml, peroxidase (horseradish) 800U/L, 4 aminoantipyrine 0.38mM, phosphate buffer, pH7.5 ± 0.1, sodium p-hydroxybenzoate 5mM, non-reactive stabilizers and fillers, sodium azide 0.1%. The spectrophotometer (Beckman, DU640 spectrophotometer) was set at a wavelength of 500 nm for detection of quinoneimine dye and concentration of glucose was calculated by linear equation using 4 standards. The principle is as follows.

1. D-glucose + H_2O+O_2 $\xrightarrow{\text{Glucose oxidase}}$ H_2O_2+D-Gluconate

2. H_2O_2+4-aminoantipyrine+hydroxybenzonate $\xrightarrow{\text{POD}}$ Quinoneimine dye+H_2O

Statistical Analysis

To determine appropriate sample size and power, G power 3.1.7 was used based on the statistics of two prior studies in which positive effects ($p < .05$) were reported. Number of subjects was determined from 10 prior experimental studies, which used short-term dietary nitrate supplementation, was also referenced (usually 9-12 subjects) (Appendix B8).

Data were analyzed using SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL). Metabolic gases (VO_2, VCO_2, and RER), mechanical performance (work done and peak torque), blood samples (glucose and lactate), and hemodynamic (blood pressure and heart rates) were analyzed by using two-way repeated-measures ANOVA (Treatment × Time) or Paired t-test. A Levene's test was used to analyze equality of variance. Significant effects from the repeated ANOVA were further explored by using an adjusted Fisher's LSD test.

To determine whether there was a difference in resting values according to NO supplementation, two-way (2×2) repeated ANOA was used. To check the differences between NO-L and PLA along the 3 different conditions (post-resting, exercise, and recovery) two-ways (2×3) repeated ANOVA was used. To compare exercise variables including work efficiency, two-tailed paired t-test was used. In addition, a two-way (2 × 4) repeated ANOVA was used to analyze comparison of sets during exercise. All results were expressed by mean ± standard error and differences were considered significant at a $p < .05$.

RESULTS

Subjects Characteristics

Seventeen subjects volunteered, but two were screened out because of low VO_{2max} values and one dropped out at the familiarization trial due to a fear of needles. A total of fourteen subjects successively completed all trials (Appendix C2). The subjects were non-smokers and had no medical history related to cardiovascular disease. BMI was 23.7 ± 0.5, VO_{2max} was 47.7 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$, and mean arterial pressure (MAP) was 90.2 ± 1.5 mmHg when they visited the laboratory for screening. Their ventilatory threshold (VT) occurred at 165 ± 9 watts while cycling and 62.4 ± 3.5 % of VO_{2max} (Table 2 and Appendix A1).

Knee Exercise Performance

In this study, the exercise was isokinetic knee contractions using a Biodex dynamometer, which were not corrected for gravity effect of limb weight.

Original unidirectional total work and peak torque values were automatically measured by the Biodex dynamometer and CSMI isokinetic software, and stored in a computer. We have organized the data into bidirectional sum for each work set and total work. To determine peak torque during the exercise trials, we used the average of the three highest torque and the three lowest torque values among the first and last six contractions, respectively (Appendix A2 to A5).

NO-L produced no significant treatment or treatment-by-time interaction effect on work done per set or total work performed (Figure 1 and Table 3). In each work set, there was a significant decrease in force production over time. The decline in work done occurred mainly between the first and second sets, and the work decline was small over the last contraction set.

During four exercise sets and six recovery contractions, peak torque (extensor peak torque: EPT, flexor peak torque: FPT) was significantly affected by exercise and recovery time (Table 4 and 5). While NO-L had no effect on knee flexion throughout the fatiguing and recovery periods (Figure 4), NO-L had a significant treatment-by-time interaction ($p = .021$) on rate of recovery, particularly between the second and third contractions ($p = .034$) (Table 4 and Figure 3). NO-L had no significant treatment effect on time to peak torque (NO-L: 0.200 ± 0.007sec vs. PLA: 0.213 ± 0.006 sec; $p = .08$) although significance was approached.

Energy Metabolism

Respiratory gas data were used to determine energy metabolism during resting, knee exercise, and recovery (Rebergs et al., 2010). The value of net oxygen consumption was used for the calculation of work efficiency (Appendix A6 to A12).

There were interaction effects ($p < .05$) of resting VO_2 and VCO_2 between pre and post treatment and these values were higher for NO-L post treatment. The post treatment comparisons (2×3 repeated ANOVA) showed respiratory gases significantly increased with exercise, and there was a significant interaction effect on VCO_2 with the value for PLA higher than NO-L. There was no interaction or treatment effect on VO_2, but there was a meaningful tendency for less oxygen to be used during the NO-L treatment. RER values were significantly lower for NO-L compared with PLA (NO-L: 1.38 ± 0.02 vs. PLA: 1.42 ± 0.02; $p = .03$ by paired t-test) (Table 6 and Figure 2).

Focusing on exercise set during exercise (2×4 repeated ANOVA), there was an interaction effect ($p = .01$) on VCO_2, specifically during the 2^{nd} exercise set ($p = .01$) with significantly lower RER values for NO-L ($p = .05$) (Table 7 and Figure 5 and 6). Oxygen

consumption increased with time even though work declined with each exercise set, and VCO_2 peaked during the 3rd exercise set and declined during the 4th exercise set. We found negative correlations ($p < .01$) between oxygen consumption and work done during knee extension exercise with no difference in slope for PLA compared with NO-L (NO-L: $r = -.546$ and PLA: $r = -.560$) (Table 8 and Figure 7 and 8). There was a significant treatment effect on total work efficiency for NO-L compared to PLA (NO-L: 2578.7 ± 132.2 ft-lb/l/min vs. PLA: 2497.5 ± 134.6 ft-lb/l/min; $p = .05$) (Table 10).

Metabolites and Hemodynamic Indicators

Resting blood lactate and glucose concentrations were not different between treatments ($p > .05$ by 2×2 repeated ANOVA). Based on data from the post treatment comparisons, there was a significant time effect ($p < .01$ by 2×3 repeated ANOVA) but no difference by treatment. Blood lactate was steeply increased with exercise and further increased during recovery, while blood glucose was increased with exercise and remained elevated during recovery (Table 9, Figure 9 and 10, and Appendix A14 and A15). Throughout the trials, there was no significant treatment effect on hemodynamic indicators such as blood pressure or heart rates (Table 11 and Appendix A16 to A18).

DISCUSSION

Through well-designed previous studies (Ignarro et al., 1987; Brown & Cooper 1994; Shen et al., 1994 & 2001; Larsen et al., 2011) we now have a better understanding of how NO mediates vascular tone and mitochondrial respiration. In addition, a number of studies have explored the nonenzymatic NO generating pathway (Weizberg & Lundberg, 1998; Govoni et al., 2008; Jansson et al., 2008), From these studies, it was found that dietary nitrates can have a positive effects on pathophysiological events as well as on performance (Cosby et al., 2003; Larsen et al., 2007; Bailey et al., 2009 & 2010; Wilkerson et al., 2012; Kelly et al., 2013; Bentley et al., 2014).

It is still unclear, however, whether NO has a positive effect on intense resistance exercise, although there have been recent human studies that suggest this is a possibility. These studies report a reduced ATP turnover rate and enhanced muscle contractile efficiency when dietary nitrates are provided (Bailey et al., 2009). In addition, Bailey et al. (2010) found that time to exhaustion during high-intensity exercise was significantly increased after nitrate administration (586 ± 80 s at PLA vs. 734 ± 109 s at NO) and decline in PCr attenuated (17.10 ± 5.34 mM at PLA 15.10 ± 4.14 mM at NO). For the reason, the present study investigated the effects of three days of dietary nitrate (NO-L) supplementation on vigorous resistance knee extension/flexion exercise performance and energy metabolism. The experimental protocol applied in this study was inspired by previous studies (Bailey et al., 2009 & 2010; Fulford et al., 2013; Larsen et al., 2011 & 2014), but we further increased the exercise intensity so that energy metabolism was primarily provided by the anaerobic energy system. This is supported by the finding that there was a significant inverse correlation ($p < .01$) found for work per exercise set

and oxygen uptake (Figure 8.1 and 8.2). That is, the fall in work with advancing exercise sets was due to a decline in anaerobic production and not due to a decline in aerobic energy production. Therefore, the present study results will address NO-L effects on intense exercise, and the action of nitrate supplementation on anaerobic energy metabolism.

Exercise Performance by Highly repeated Isokinetic Concentric Knee Contractions

Isokinetic technology has in use to evaluate the individual muscle function such as strength and power for elite athlete as well the general population since the late 1960s (Hislop & Perrine, 1967) and is regarded as an efficient and safe form of assessment because the resistance is accommodated to the force expressed throughout the range of motion (Wyatt et al., 1981). Isokinetic concentric measurement at high-speed angular velocity (180°/sec) is normally used to test muscle power. Youn (1997) suggested that highly repeated isokinetic contractions can be used for evaluation of endurance performance, and Kim (2009) confirmed that 30 seconds of isokinetic knee contractions is highly correlated with the Wingate fatigue ratio. In addition, highly repeated knee exercise (one minutes at high angular speed) has been proposed to test the anaerobic energy system (Douris, 1993) appropriately. For the current study, we designed an exercise protocol of four sets of twenty-eight knee extension/flexion contractions at 180°/sec speed with each set lasting 30 seconds and with 30 seconds rest between sets. Recovery protocol was monitored by performing maximal knee extension/flexion contraction every 120 seconds over 12 minutes. This allowed the evaluation of (i) peak torque (ii) muscle endurance, and rate of recovery.

The prominent finding was that extensor peak torque recovery speed was meaningfully faster for NO-L ($p = .09$). Treatment by time analysis indicated that the peak torque for the third

recovery contraction was had reached 80% of initial peak torque and significantly greater than PLA ($p = .034$). This positive recovery effect is remarkable when considering the first extensor peak torque was 1.6% higher for NO-L and more work done during NO-L (11453 ft-lb at PLA vs. 11512 ft-lb at NO-L). Therefore, it can be said that NO-L treatment had a positive effect on extensor power recovery from the exhausting exercise. It is also noteworthy that time to peak torque for NO-L was significantly shorter for both extension and flexion during exercise (Extensor: 0.213 ± 0.006 sec at PLA vs. 0.200 ± 0.007 sec at NO-L; $p = .08$ & Flexor: 0.218 ± 0.008 sec at PLA vs. 0.209 ± 0.008 sec at NO-L; $p = .22$). Time to peak torque for knee extensor at a speed of 180°/sec occurs between 60° and 50°, when the range of motion is 90° (Knapik et al., 1983) and time-wise occurs between 0.166 sec to 0.222 sec. Time to peak torque is used to evaluate rate of force production and a delayed time to peak torque suggests a reduced recruitment of type II fibers (Kannus, 1994). Since NO-L extensor and flexor times to peak torque were superior to PLA during all exercise sets (Appendix A5, extensor $p = .04$ and flexor $p = 0.11$) it is possible that three days of NO-L may improve motor unit recruitment during resistance exercise, particularly in fast twitch muscles.

Energy Metabolism

Another noticeable finding was that there was a significant difference in RER during repeated isokinetic exercise (1.42 ± 0.02 at PLA vs. 1.38 ± 0.02 at NO-L; $p = .03$). In general, RER represents the ratio between carbohydrate and fat oxidations during submaximal exercise (Ivy et al., 1979), whereas during server exercise when RER exceeds 1.0, it affected by the buffering of hydrogen ions (Wasserman, 1987). The imbalance between hydrogen ion generation and removal is one of the reasons causing muscle contraction disturbance (Gladden, 2004). The

significantly lower RER found for the NO-L treatment seemed to be accomplished by the combine effect of a little less oxygen consumption and much less carbon dioxide production (Table 6 and 7). These results are interesting considering there was slightly more work and less lactate production during NO-L exercise trial compared with PLA (Table 7 & Figure 1 and 9). Collectively, it can be surmised that there was lower metabolic rate in the nitrate supplementation trial. This result is in line with recent human studies (Engan et al., 2012; Larsen et al., 2014), which reported a lower metabolic rate during apnea and resting after nitrate supplementation. Two reasonable explanations include a reduced ATP turnover as suggested by Bailey et al. (2009 & 2010) and more efficient mitochondria as suggested by Larsen et al (2011). Our results, however, do not support a reduced ATP cost of exercise since there was no difference in work between treatments for the third ($p = .40$) or fourth ($p = .51$) exercise set or total work performed (11453.2 ± 379.8 ft-lb at PLA vs. 11511.8 ± 339.4 ft-lb at LO-L; $p = .66$). If NO-L was reducing the ATP turnover, it seems reasonable that work would be greater for NO-L from the second set to the end or total work would be meaningfully higher for NO-L than PLA. However, there was only slightly more work done during NO-L and during the second exercise set, there was 29 ft-lb less work for NO-L compared with PLA. Instead, our results suggest a more efficient oxidative phosphorylation process. Larsen et al. (2011) claimed that short-term nitrate supplementation lowered the expression of proton channel proteins and the saved protons increased the ATPase driving force and thus ATP production. Thus, in our study a slightly reduced oxygen cost combined with slightly more total work was connected to an enhanced work efficiency ($p = .05$). It is suggested that additional research be conducted to determine if dietary nitrate can improve aerobic metabolic efficiency during intense exercise.

Blood Pressure

The best-known role of NO in the mammalian body is the regulation of vascular tone (Ignarro et al., 1987). In this regard, dietary inorganic nitrate supplementations have been reported to help lower resting blood pressure in healthy adults (Larsen et al., 2006; Webb et al., 2008), elderly people (Kelly et al., 2013) and aged people with reduced functional hemodynamics (Kenjale et al., 2011). This demonstrates the bioactive form of nitrite works on smooth muscle relaxation in both normoxic and hypoxic internal body conditions. Considering exogenous NO donor is less effective than enzymatic NO production (Xia et al., 1996), however, and there are more NOS activators such as acetylcholine and bradykinin (Pearson & Vanhoutte, 1993; Victor et al., 2009), the effects of dietary nitrate is not small in terms of a therapeutic response. The effect of dietary nitrate on vascular tone may also provide a reason why vegetarians have a lower risk of the cardiovascular disease (Lundberg & Weitzberg, 2010).

In the current study, there was no significant treatment difference resting mean arterial pressure (MAP) ($p = .45$). This is not in agreement with previous studies and we speculate several possibilities to explain the current result. First, it is unlike a reliable resting blood pressure can be made prior to exercise and when undergoing verbal communication (Long et al., 1982), limbs positioning (Beevers et al., 2001), and possibly anticipating the exercise to come (Handler, 2009). Therefore, to get reliable data of the resting blood pressure, repeated measurement and averaging is required. Webb et al. (2008) and Kelly et al. (2013) used averaged values, two out of three and three out of four time measurements, respectively. In this study, we tried to identically match circadian rhythm, room temperature, resting interval, measurement position, and inter-rater reliability between trials, but we did do multiple measurements. Second, we confirmed that different positioning resulted in blood pressure changes. Based on these pilot

results, we decided to measure blood pressure on the Biodex machine while the subjects were wearing metabolic equipment such as head set and mouth peace (Appendix B4). On the other hand, pre-treatment resting blood pressure measurements were taken while the subjects were seated on normal chair with feet on the floor and the arm supported on a table. Subjects wore no equipment during the pre-blood pressure measurements. Comparing MAP from those two positions, we saw a big change occurred between the two different positions with Biodex position increasing MAP significantly ($p < 0.01$) regardless of treatment. Third, the measurement timing could have been the reason for not finding a treatment effect. Unlike preceding NO researches in which approximately two and a half hours is the average timing to initiate blood pressure measurement (Vanhatalo et al., 2010; Kelly et al., 2013) or to see the significant difference (Webb et al., 2008; Kenjale et al., 2011), we checked the resting blood pressure around 37 minutes (Appendix C5) after each treatment. Possibly this short interval did not provide enough time to see the bioactive nitrite effect on resting MAP. Increased blood nitrite acts as a bioactive precursor for NOS-independent nitric oxide generation (Lundberg & Govoni, 2004) and the nitrite peak level is known to occur around at two and a half hours after acute supplementation (Wylie et al., 2013A).

One concern about the nitrate effect on blood pressure is whether this is an acute or chronic effect. Almost all researches have reported positive effect after two and a half hours of supplementation regardless of whether the supplement was provided acutely or chronically (Webb et al., 2008; Vanhatalo et al., 2010; Kenjale et al., 2011), and there does not appear to be any difference in blood pressure response between acute and chronic supplementation (Webb et al., 2008). So it seems that boosted blood nitrite results in a positive effect on resting blood

pressure and the impact appears to be acute in nature but needs to be confirmed with varied study designs.

Conclusion

Considering the increase number of individuals participating in resistance exercise, and the lack of dietary nitrate studies investigating its impact on intense resistance exercise, the current study is noteworthy because (i) it investigated the utilization of the anaerobic energy system, and (ii) shorten the loading interval between supplementation and exercise to within forty minutes. The study results suggested that three days of lozenge dietary nitrate has a positive effect on intense resistance exercise performance by enhancing knee extension time to peak torque and rate of recovery, and increased work efficiency.

REVIEW OF LITERATURE

Nitric Oxide Chemical Properties

Nitric oxide (NO) is a small gas compound of a single nitrogen and oxygen atom with a molecular weight of 30.01 gram per mole. It acts as an important cell-signaling molecule in the mammalian body due to several distinctive chemical characteristics.

NO can pass through the cell membrane without support of specialized transporter proteins because of its small size and uncharged polarity (Pearson & Vanhoutte, 1993). This property allows NO to mediate cell functions not only where it is generated but also where it is adjacent. Because the NO transportation complies with simple diffusion, NO also moves along a concentration gradient (Toledo & Augusto, 2012).

As a free radical, the unstable electric configuration of NO's outer orbital-layer is the main reason NO easily interacts with many other chemical species. Since NO has a high affinity for metal elements like iron and copper, NO rapidly reacts with ferrous iron (Fe2+), Cu^{+}, free iron, and the iron site of iron-sulfur centers and heme-proteins with liberal ligand place (Cooper, 1999). In this regard, soluble guanylate cyclase, cytochrome oxidase, and hemoglobin, which are well known for typical heme-containing enzymes, would be the main target proteins that directly react with NO. (Kelm, 1999; Moncada, 2000).

Based on its short half-life (milliseconds to seconds depending on concentrations of scavenging proteins), NO-mediated physiological effects are transient and rapid. Since it also cannot be stored in intact form in many cases, it is normally metabolized to oxyanion forms, which are regarded as potential NO sources, like nitrite and nitrate, or transformed to its derivative forms whose mediation is more likely to induce structural change and prolonged

effects, such as nitrogen oxide, peroxynitrite, and nitrosothiol (Hakim et al., 1996; Hord et al., 2009; Dudzinski et al., 2006; Tengan et al., 2012).

NO Research Area

NO is mainly produced by NO generation from the family of nitric oxide synthase enzymes (NOSs), which are distributed from the brain to the distal limb muscles. It is, therefore, not surprising that NO mediates various pathophysiological events throughout the whole body. Based on the study results using a plethora of interventions for NO production, the importance of NO-mediated biological effects in various systems have been investigated (Hofmann et al., 2006).

NO plays key roles in neural signaling for learning and memory (Contestabile et al., 2003) as well as normal sexual function (Meldrum et al., 2012). It facilitates cell or tissue defense mechanisms against infection and inflammation (Nathan & Hibbs, 1991) by regulating the immune system. NO mediates glucose metabolism (Bradley et al., 1999) and mitochondrial functions (Shen et al., 2001) in the muscular system, and is a powerful vasodilator, and regulates vasomotor tone and blood pressure (Moncada & Higgs, 1993; Webb, 2003).

Since the critical confirmation that the physiological function of NO and endothelium-derived relaxing factor (EDRF) are actually not different (Ignarro et al., 1987), a number of exercise studies have been conducted.

Considering the importance of optimal supply of energy sources and recovery materials to the skeletal muscle during and after exercise (Stamler & Meissner, 2001), NO's role on regulation of vasomotor tone and thus blood flow has become an attractive area of investigation in the exercise science field. It is also known that NO influences mitochondrial respiration and oxidative phosphorylation efficiency (Clerc et al., 2007; Larsen et al., 2011A) as well as

mitochondrial biogenesis (Nisoli & Carruba, 2006). In addition, recent evidences suggest that NO affects the ATP turnover rate (Bailey et al., 2010) and contractile function (Hernandez et al., 2012) of skeletal muscle. Therefore, it seems appropriate to examine its mechanisms of action for facilitating physiological events with respect to exercise performance.

Nitric Oxide Mechanism of Action on Vasodilation

Vasodilation is consequence of Vascular Smooth Muscle Relaxation

Vasodilation results from the relaxation of vascular smooth muscle tissue, which encloses blood vessels that are composed of a single layer of endothelial cells. Unlike skeletal muscle, smooth muscle contraction depends on the phosphorylation states of myosin light chain (MLC). The phosphorylation of MLC in the smooth muscle leads to contract and dephosphorylation leads to its relaxation.

This unique contractile characteristic of relaxation with dephosphorylation of the MLC is caused by (i) reduced affinity between the myosin head and myosin-binding site on the actin filament, as well as a weak myosin light chain coupled inactivated myosin ATPase, and (ii) the lack of troponin and tropomyosin, which means the myosin-binding site is always open and thus there is no need for calcium-activated tropomyosin conformational change for starting cross-bridge cycling.

Two enzymes, myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), regulate MLC phosphorylation status and the catalytic activities are modulated primarily by cytosol calcium concentration $[Ca^{2+}]_i$.

In a different sense, cytosolic [Ca^{2+}] plays a key role in smooth muscle contraction and relaxation in that it is a critical factor for determining the downstream effect of Ca^{2+}-calmodulin (CaM, calcium-binding messenger protein) complex.

Elevated Ca^{2+}-CaM complex directly promotes MLCK activity (Hofmann et al., 2006). As a Ca^{2+}-CaM dependent enzyme, MLCK phosphorylates 20-kDa myosin light chain on serine residues and thereby potentiates myosin ATPase. This then results in muscle contraction, whereas MLCP leads to the reverse reaction when the cytosol calcium level falls (Webb, 2003).

RhoA (ras homolog gene family member, small GTPase protein)/Rho kinase (ras associated protein kinase) is another [Ca^{2+}] sensitizing pathway that regulates MLC activity. Rho kinase phosphorylates myosin-binding site of MLCP and inhibits MLCP activity. This then indirectly helps MLCK to maintain phosphorylation of MLC during smooth muscle contraction (Webb, 2003). Therefore, without high level of calcium influx, cross bridge cycling remains in the inactive state.

NO mediates Vasodilation through cGKI activity

NO acts as a potent factor of vascular smooth muscle (VSM) relaxation in that NO is able to control both Ca^{2+}-CaM and RhoA/Rho kinase induced routes by elevating the cyclic GMP dependent protein kinase (cGKI) level. This then affects many downstream activities (Hofmann et al., 2006; Pearson &Vanhoutte, 1993).

NO-related vasodilation is initiated with the simple diffusion NO into neighboring smooth muscles. When NO diffuse into the cytoplasm of VSM it binds to an iron-containing site found on soluble guanylate cyclase (sGC) and activates the enzyme activity. Activated sGC facilitates the hydrolysis of guanosine triphosphate (GTP) followed by increasing the level of

cyclic guanosine monophosphate (cGMP). Concentrated cGMP, as a second messenger, turns on the chain reaction of cGKI, which is a serine/threonine kinase. cGKI, also known as protein kinase G (PKG), is expressed in high level in vascular smooth muscle (Geiselhoringer et al., 2004).

Elevated cGKI activity lowers $[Ca^{2+}]_i$ through (i) phosphorylating and activating sarcoplasmic/endoplasmic reticulum Ca^{2+}-Mg^{+}-ATPase (SERCA), which promotes calcium reuptake into the sarcoplasmic reticulum (SR) (ii) phosphorylating or indirectly regulating large-conductance calcium-activated potassium channels (BKca), which elevates calcium efflux to the extracellular matrix (iii) and thereby leading to hyperpolarization inside of the cell and inhibiting voltage-dependent calcium channel (L-type calcium channel). This prevents calcium influx to the cytosol (Hofmann, 2006). Reducing availability of $[Ca^{2+}]_i$ leads to reduced Ca^{2+}-CaM complex and thus restores MLCK into its inactivated dephosphorylated state.

Moreover, activated cGKI directly activates MLCP activity through the interaction with MYPT1, myosin phosphatase target subunit 1 or myosin-binding subunit of MLCP and thus dephosphorylates MLC. It also deactivates phospholipase C activity and inositol triphosphate (IP3) synthesis, which act as an intermediate signaling of SR calcium release. It may reduce RhoA/Rho kinase action by decreasing GTP availability for RhoGEF (Webb, 2003; Moncada & Higgs, 1993; Cohen et al., 1998).

NO Action Mechanism on Mitochondrial Functions

As a terminal enzyme of electron transport chain (ETC), cytochrome c oxidase, contributes to electron removing and partly to the electrochemical gradient between the intermembrane space and matrix. O_2 transiently binds to the Fe^{2+}at ferrous-copper (Fe^{2+}-CU^{+})

binuclear site of cytochrome c oxidase, which takes a total four electrons transferred from the series of respiratory enzymes releasing water and heat (Moncada, 2000; Brown, 2001).

Normal activity of cytochrome oxidase for mitochondrial respiration accounts for ~90% of systemic oxygen consumption of mammalian metabolism (Babcock & Wikstrom, 1992). Since the O_2 amount consumed by the mitochondrial respiration is closely related to the production rate of cell-usable energy, ATP, it seems reasonable that oxygen consumed at resting or during at least submaximal exercise should be matched with the energy produced. For some reasons, however, oxygen utilization through the respiratory enzymes is not precisely coupled with oxidative phosphorylation.

In this case, NO mediations on mitochondrial functions may provide the clue or explain the mismatching (Brand et al., 1999). Through the results of the studies using isolated cell and mitochondria of astrocytes, hepatocytes, and myocytes in vitro (Cleeter et al., 1994) or animals in vivo (Shen et al., 1994), we now know that NO interaction with various mitochondrial sub-particles cause the mitochondria to function more precisely.

NO Reversibly inhibits Mitochondrial Respiratory Function

The best-known NO intervention on mitochondrial metabolism is that NO reversibly inhibits the cytochrome c oxidase activity by competing with the oxygen molecule on the Fe^{2+}-CU^{+} binuclear center and thus leads mitochondria to use less oxygen (Boveris et al., 1999). Physiological nitric oxide level (0.1nM-100nM) (Bellamy et al., 2002) may inhibit this key respiratory enzyme in a non-directional manner resulting in [NO]-dependence (Cleeter et al., 1994; Brown and Cooper, 1994) and [O_2]-dependence inhibition (Poderoso et al., 1996; Brown, 1999).

Reversible NO inhibition is influenced by the redox state of this enzyme. Unlike O_2, NO is able to bind both oxidized and reduced forms of cytochrome oxidase (Taylor & Moncada, 2010). In a high [O_2] surrounding environment (oxidized form of cytochrome oxidase, Fe^{3+}-CU^{2+}), NO binds to Cu^{2+} without competing with O_2 and is instead metabolized to nitrite (NO_2^-) maintaining normal function of respiratory enzymes, but under low [O_2] tension (reduced form of cytochrome oxidase, Fe^{2+}- CU^+), NO binds to ferrous (Fe^{2+}) iron competing with O_2, decreasing oxygen consumption, and increasing reactive oxygen species (ROS), and thus producing a toxicity sequence of events (Cooper & Brown 2008).

It should be mentioned that the duration and degree of NO effects on mitochondrial subcomponents is tightly depends on the oxygen concentration (Takehara et al., 1995; Koivisto et al., 1997). Effective respiratory half inhibition of [NO]/[O_2] ratio was achieved at [60nM] /[30µM] and at [270nM]/[145µM] (Brown & Cooper, 1994). Considering the normal oxygen concentration is in the range of 20-50µM for cytosol and 1-5µM for mitochondria (Takehara et al., 1995), the basal NO level (0.1nM-100nM) may already have crucial effects on mitochondrial respiratory function. However, in a normoxic situation reversible inhibition may not cause significant reduction of respiratory function because the superfluity of cytochrome oxidase also provides oxygen with buffer zone up to a critical point of [NO]/[O_2] (Palacios-Callnder et al., 2007). A significant question is does the reversible inhibition reduce ATP production?

NO Irreversibly mediates Mitochondrial Respiratory Function

High concentrations of [NO] or [NO]/[O_2] ratio tend to retain the reactions with superoxide (O_2^-), the iron-sulphur (Fe-S) of complex reductases, and reduced cytochrome oxidase. As a result, by-products such as peroxinitrite ($ONOO^-$) and S-nitrosothiols (RSNO) are

produced. These derivative forms, which are regarded as more stable and also cell damageable (Hibbs et al., 1988; Brown, 1997), allow NO to indirectly inhibit the respiratory enzymes (Poderoso et al., 1996) in an irreversible manner.

Activated macrophages or cells, which are part of the inflammation process, provoke iNOS enzyme activity by using stimulators like cytokine or endotoxin (Moncada & Higgs, 1993). NO released from iNOS activity is accumulated over the basal level and sustained until the stimulations are removed.

At a diffusion limited rate, NO rapidly interacts with superoxide and produces peroxynitrite (Heales et al., 1994). Via an oxidizing reaction, peroxynitrite can inhibit mitochondrial respiration in an irreversible manner and also damage mitochondrial complexes I and II, the ATP synthase, the mitochondrial membrane, and superoxide dismutase (Brown, 1999; Cassina & Radi, 1996; Lizasoain et al., 1996; Brookes et al., 1998). In addition, peroxynitrite or high levels of NO (>1μM) may irreversibly inhibit cytochrome oxidase and thereby raise its K_m for oxygen (Sharpe et al., 1998).

NO interaction with thiols or protein cysteine residues indirectly occurs (Patel et al., 1999; Gaston, 1999) and may cause damage on iron-sulfur clusters and released iron from the center (Tengan et al., 2012). Nitrosylation of essential thiols in complex I (Clementi et al., 1998) inhibits the intact function of the enzyme and this inhibition may depend on glutathione levels. Inhibition of complex I is associated with decreased activity of complexes II, III, IV (Bolanos et al., 1996). Furthermore, inhibition of complex III excessively increases O_2^- and thus hydrogen peroxide (H_2O_2), which is also involved in oxidative damage (Moncada, 2000).

Impaired activity of mitochondrial intermediate enzymes can cause poor energy metabolism due to limited electron transferring coupled with less proton efflux and thus reduced

ATP production. This then may have significant implication on physical activity issues that would evoke anaerobic metabolism. In fact, Brookes et al. (1999) confirmed the hypothesis that NO inhibition of mitochondrial respiration will reduce ATP generation by using an isolated brain mitochondrial preparation. After adding 4μM NO, ATP production was immediately diminished and then recovered up to 70% of control. This result suggested that pathologically induced high levels of NO inhibits the mitochondrial function of ATP production and causes irreversible damage to inner membrane enzymes.

NO modulates Proton Leak Channels and thus regulate P/O Ratio

Oxidative phosphorylation efficiency is typically represented by the P/O ratio (Larsen et al., 2011). The P/O ratio can be calculated by the rate of ATP produced per oxygen consumed during rapid respiration; state 3, in which unlimited oxidizable substrates and ADP allow ATP synthase to produce ATP. In this process, proton influx into the matrix provides the driving force of the F0/F1 turbine (Noji et al., 1997). During slower rates of oxygen consumption such as state 4, after ADP depletion or after adding an ATP synthase blocker like oligomycin (Rolfe et al., 1999) allows the testing of proton leak kinetics. Proton leaks, which include uncoupling proteins (UCPs) and adenine nucleotide translocase (ANT) across the inner membrane may account for up to 20% of estimated in vivo standard metabolic rate (Rolfe et al., 1999). Divakaruni & Brand (2011) suggest that "futile" proton cycling may prevent the host from oxidative damage connected to aging or degenerative disorders although state 4-respiration is not necessary for ATP production. Considering skeletal muscle metabolism, it seems partly right because oxidative phosphorylation is precisely coupled with the energy requirement (Atkinson & Walton,

1967; Larsen et al., 2011). Technically, to improve the P/O ratio, it is better to (i) provide NADH-linked substrates, and (ii) reduce proton leaks (Clerc et al., 2007).

Extra NO may have a significant effect on proton leaks via a short-term manner and thus affect P/O ratio. In a human study, Larsen et al. (2011) confirmed that there was a significant down-regulated expression of ANT ($P = .01$), which is profound in skeletal muscle, and UCP3 ($P = .17$) after 3 days of sodium nitrate supplementation. As a result, there was significantly less state 4 respiration, and improved P/O ratio with the treatment group showing a reduced proton leakage and thus improving the driving force at ATP synthase. Coincidently, there was less ($P = .02$) whole body oxygen consumption at 50% VO_{2max} during submaximal cycling demonstrating an increase in oxidative phosphorylation efficiency. There was speculation that the cell sensed mild hypoxic conditions caused by nitrite, and thus NO exposure over a prolonged period of time may elicit signaling pathways to down-regulate ANT. However, exactly how short-term nitrate supplementation affects the expression on mitochondrial uncoupling proteins remained to be further investigated.

Shen et al, (1994) were the first to investigate the importance of basal NO on mitochondrial metabolism. Their research was the first in vivo study to test whether basal NO levels modulate whole body oxygen consumption and tissue oxygen extraction. At resting energy requirement, conscious dogs were administrated NLA (L-arginine competitor), which caused an increase in tissue oxygen extraction ($p < .01$) and oxygen consumption ($p < .05$) compared to control levels despite worse hemodynamic indications. This result may indicate the crucial role of basal NO in regulating hemodynamics and tissue metabolism; indicate that the down-regulated expression of proton leak channels is not only mechanism by which mitochondrial efficiency in improved. Interestingly, it was found body temperature of the dogs was increased

after NO inhibition and indicates that NO's regulation of oxygen consumption is related to thermodynamics. The result also showed indirectly that there was an improved P/O ratio because the same resting position and identical experimental conditions were provided for both treatments. In another study using isolated guinea pig hearts, Shen et al, (2001) directly demonstrated that basal NO improved the P/O ratio by showing reduced oxygen consumption, but not ATP production.

Therefore, it can be said that physiological levels of NO already has a significant role in regulating both the mitochondrial respiratory chain and proton dissipation channels. May be that basal NO might already inhibit electron transferring and proton pumping since state 4 respiration disproportionately increases when the proton gradient raise (Nobes et al., 1990). Collectively, the mechanism for how NO improves the P/O ratio seems more related to inhibition of the proton leakage. This would (i) increase potential energy to driving ATP production, and (ii) better regulate heat production.

Nitric Oxide Generation Passageways

Under physiological conditions, basal NO supply is mainly accomplished by NO production from nitric oxide synthase (NOS), which is placed at specific cellular and tissue locations (Alderton et al., 2001). In eukaryotic cells, typically, NOS has three well-known isoforms: nNOS (neuronal NOS or type I NOS), eNOS (endothelial NOS or type III NOS), and iNOS (inducible NOS or type II NOS) (Stuehr et al., 1999). nNOS and eNOS are responsible for constitutive NO generation in the physiological condition and their activities are calcium dependent, whereas iNOS is responsible of inducible NO production in under pathological conditions and its activity is calcium independent (Lin et al., 2004).

Structurally, NOS contains two, an oxygenase and reductase domain. The oxygenase domain consists of the binding sites for L-arginine, heme (Fe), and tetrahydrobiopterin (BH4), whereas the reductase domain contains the binding sites for flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide phosphate (NADPH), and calmodulin (Alderton et al., 2001). The overall reaction is the movement of electrons from the reductase to the oxidase domain. NADPH acts as the initial electron donor, FAD and FMN as intermediate electron acceptors, heme and BH4 as intermediate electron acceptors in the oxidase domain. Arginine and O_2 together are the final electron acceptors. Activation of calcium-calmoduin facilitates this reaction of nNOS and eNOS, but is not required for iNOS activity (Alderton et al., 2001). As a result, L-arginine and O_2 are transformed to L-citrullin and NO (Bescos et al., 2012).

It is also well known that NO generation can takes place via a nonenzymatic route in which NO production results from chemical reduction of nitrite. Instead of using NOS enzyme, this route depends on nitrate or nitrite reductase activity (Weitzberg & Lundberg, 1998). In this case, inorganic nitrate, which is rich in green leafy vegetables and beetroot, can serve as precursors for nonenzymatic NO production (Hord et al., 2009).

In the initial step, nitrate ingested into the intestines is taken up in the circulatory system and delivered to the salivary glands of the mouth where nitrate is converted to nitrite by commensal bacteria (Jansson et al., 2008). The nitrite is reduced to NO through different pathways including deoxy-hemoglobin and myoglobin (Lundberg et al., 2008). Nitrite reduction to nitric oxide is preferred under hypoxic conditions (Jensen, 2009) due to increased reaction availability of the well-known nitrite reductases such as deoxy-hemoglobin, deoxy-myoglobin, xanthine oxidoreductase, and protons (Lundbergy et al., 2008). Indeed, in the study of isolated

perfused rat heart with ischemia-reperfusion injury (Zweier et al., 1995), there was a 10-fold increase in NO production, but it was only partially inhibited by L-NAME infusion showing that the NO production route was nonenzymatic. The fact that an internal environment of low pH and low [O_2] caused by ischemia or hypoxia is optimal for nitrite to be converted to nitric oxide (Lundberg, 2008) indicated several potential applications for the NOS independent pathway under pathophysiological conditions.

Compared to rodents, humans have very lower nitrate reductase activity (Jansson et al., 2008). Nitrate concentration was found to be10-20 fold higher in saliva than blood suggesting that oral bacteria is the main way nitrate is converted to nitrite (Lundberg et al., 2004). Exogenous nitrate supplementation or diet can be converted to bioactive form of nitrite released with limit endogenous nitrate reductase activity.

Nitrate Supplementation Improves Exercise Performance

Vanhatalo et al. (2010) examined the acute and chronic effects of dietary nitrate supplementation on moderate intensity constant cycle exercise and found a lower oxygen cost at the same exercise intensity. Bescos et al. (2011) tested a single dose of inorganic nitrate supplementation and found that nitrate supplementation significantly reduced VO_{2max} ($p = .01$) and VO_2 at a standardized power output ($p = .03$). Cermak et al. (2012) found that 6days of dietary nitrate supplementation in the form of beetroot juice (~0.5 L/day) reduced VO_2 during sub-maximal exercise and increased tolerance to high-intensity work rates. This suggested that nitrate can be a potent ergogenic aid. They also suggested that the mechanism responsible for a lower oxygen uptake during exercise is likely related to the role of nitrite and nitric oxide as regulators of cellular oxygen utilization. Kelly et al. (2013) investigated the effects of short-term

dietary nitrate supplementation and found enhanced VO_2 kinetics in the supplement group. Larsen et al. (2011) explained that reduced oxygen cost and enhanced metabolism efficiency results from an enhanced P/O ratio. This was in part due to a reduced expression ANT, which reduced proton leakage. Lansley et al. (2011A) found that acute dietary nitrate supplementation improved 4km and 16km cycling time trial performance in competitive cyclists by 2.8% and 2.7%, respectively. The improved time trial performance after ingestion of nitrate-rich beetroot juice was subsequent to a significantly greater power output for the same oxygen consumption. Similar effects were shown in different type of exercise. Fulford et al. (2013) investigated the effects of dietary nitrate supplementation on resistance exercise performance and found that a significant reduction in PCr cost per unit force output in the NO treatment group (Table 1).

Summary

From vasodilation to mitochondrial function NO mediates many cell-signaling pathways. Its mechanism of action is normally accomplished by redox or nitrosative reactions (Andrade et all, 1998). NO mediates vasodilation by increasing cGMP dependent protein kinase activity. This reduces [Ca2+] and RhoA/Rho kinase activity and thus results in the dephosphorylation of MLC. NO also mediates mitochondrial function related to energy metabolism. NO inhibits respiratory enzyme proteins and reduces its oxygen utilization both in a reversible and irreversible manner. NO also regulates proton leakage by reducing the expression of ANT or UCPs, and this contributes to enhanced energy efficiency. Two NO production pathways exists. Nitric oxide synthase is the main route generating NO and plays a key role in mammalian tissue under normal conditions. eNOS and nNOS are responsible for constitutive NO production, whereas iNOS is in charge of inducible NO production among the three isoenzymes. The discovery of a

nonenzymatic NO production pathway provided a novel way to generate NO for therapeutic or athletic endeavors, but the lack of a reductase, nitrate converting enzyme in manuals can limit use of this pathway if one does not have the proper commensal bacteria to reduce nitrate to nitrite. However, in recent years many exercise studies have been conducted to the effects of nitrate supplementation and found it to have a positive effect on performance.

Tables

TABLE 1. Nitrate Supplementation Effects on Exercise Performance

Author (Years)	**Subjects**	**Supplementation and exercise**	**Significant results**
Larsen FJ (2007)	. 9 males . 28±6 years . Well-trained cyclists	. 3days, 0.1 mmol/kg/day of sodium nitrate at 1h before the test . Incremental cycling test (~35min)	. ↑plasma nitrite . ↓VO_2 during sub-maximal exercise
Bailey SJ (2009)	. 9 males . 26±6 years . Recreationally active	. 6 days, ~11mmol nitrate 00ml beverage . 20w to moderate – 20 min interval – high intensity cycle exercise	. ↑plasma nitrite, ↓systolic blood pressure . ↓VO_2 cost at moderate . ↓VO_2 slow component amplitude at high exercise, ↑time to task failure
Bescós R (2011)	. 11 males . 34.3±4.8 years . Cyclists	. Acute, 10mg/kg body wt. at 3h before the test . Incremental cycling	. ↑plasma nitrate and nitrite . ↓VO_2 peak . ↓VO_2: power output
Lansley KE (2011)	. 9 males . 28±4 years . Club level cyclists	. Acute, 6.2mmol of nitrate in 500ml beetroot juice 2.5 h prior to exercise test . 4km and 16km cycling time trials	. ↑plasma nitrite . ↑time trial performance
Larsen FJ (2011)	. 11 males 3 females . 25±1 years . Healthy nonsmoking	. 3 days, 0.1mmol/kg/day NaNO3 at 1.5h before the test . Sub-maximal cycling (100, 120, or 150 w) until steady state	. ↑plasma nitrate and nitrite . ↑P/O ratio and maximal ATP production . ↓leak respiration
Vanhatalo A (2011)	. 7 males 2 females . 28±7 years . Recreationally trained	. 1day, 750ml beetroot juice (9.3mmol nitrate) at 24h, 12h, and 2.5h prior to exercise test . Knee extension (~8min)	. ↑plasma nitrite . ↓PCr recovery time . ↑task failure time

TABLE 1. Nitrate Supplementation Effects on Exercise Performance

Cermak NM (2012)	. 12 males . 31±3 years . Cyclists	. 6 days, ~8 mmol/d nitrate as 140 ml beetroot juice at 2.5 h before the test . 60min sub-maximal cycling and 10km time trial	. ↑plasma nitrate . ↑time trial performance . ↓ sub-maximal oxygen cost
Masschlein E (2012)	. 15 males . 21.1±1.0 years . Healthy active non-smokers	. 6 days, 500ml beetroot juice (0.07mmol nitrate/kg) . 20 min 45% VO_2 peak and incremental cycling	. ↑plasma nitrate and nitrite . ↓oxygen cost at rest and 45% exercise . ↑arterial O_2 saturation
Wilkerson DP (2012)	. 8 males . 31±11 years . Club level cyclists	. Acute, 6.2mmol nitrate beetroot juice . 50 mile time trial	. ↑plasma nitrite . ↑correlation b/w nitrite and reduction time trial completion time
Fulford J (2013)	. 8 males . 24±4 years . Recreationally trained	. 15 days, 10.2mmol nitrate as 250×2 beetroot juice at 2.5 h before the test . Maximal voluntary isometric knee muscle contractions (~10 min)	. ↑plasma nitrate . ↓PCr cost
Pinna M (2014)	.14 males . 34.7±7.5 years . Moderately trained . Swimmers	. 6 days, beetroot juice containing 5.5 mmol nitrate . Incremental swimming test	. ↑workload at anaerobic threshold . ↓aerobic energy cost
Molecular Weight; NO: 30.01g/mol // NO_2^-: 46.0055g/mol // $NO3^-$: 62.0049g/mol // $NaNO_2$: 68.9953g/mol // NaNO3 : 84.9947g/mol			

TABLE 2. Subject Characteristics (N=14, Mean ± Standard Error)

Age (yr)	Height (m)	Weight (kg)	BMI	VO_{2max} (l/min)	VO_{2max} (ml/kg/min)	HR_{max} (bpm)	MAP (mmHg)	VT (l/min)	VT (watt)
22.71	1.75	72.93	23.71	3.45	47.67	188.21	90.17	2.15	165.36
±0.67	±0.02	±2.18	±0.52	±0.10	±1.44	±3.11	±1.42	±0.01	±8.70

Subjects were non-smokers and had no history of cardiovascular disease. They regularly performed cardio or resistance type workouts two to three times a week. VO_{2max} values averaged over 20 seconds. In order to determine ventilatory threshold (VT), VCO_2vs VO_2 method was used.

TABLE 3.Total Work (ft-lb), (Mean ± Standard Error)

Treatment	W 1^{st} set	W 2^{nd} set	W 3^{rd} set	W 4^{th} set	W Total
NO-L	4027.50	2800.50	2404.50	2279.29	11511.79
	±129.29	±100.55	±88.62	±93.67	±339.38
PLA	4007.43	2829.36	2363.14	2253.29	11453.21
	±116.98	±124.07	±99.95	±92.15	±379.84

Data represent work of the dominant leg. Each exercise set consisted of 28 repetitions of extensor and flexor work, and total work was the sum of all 4 sets.

TABLE 4. Extension Peak Torque (ft-lb), (Mean ± Standard Error)

	Exercise (1~4)								Recovery (5~10) ‡					
Treatment	EPT 1H	EPT 2H	EPT 3H	EPT 4H	EPT 1L	EPT 2L	EPT 3L	EPT 4L	EPT 5	EPT 6	EPT 7	EPT 8	EPT 9	EPT 10
NO-L	108.08	85.56	74.45	70.88	60.12	36.66	31.19	29.92	60.71	74.29	87.07	88.29	91.93	92.79
	±3.22	±2.81	±3.22	±3.27	±1.69	±1.77	±1.75	±1.83	±5.99	±6.25	±5.96	±4.94	±4.76	±4.11
PLA	106.37	87.66	74.00	72.09	60.01	35.42	31.55	30.31	61.93	77.36	81.79	90.00	89.71	86.29
	±3.74	±3.07	±3.25	±3.31	±2.05	±1.95	±2.13	±1.76	±6.16	±5.93	±5.81	±4.46	±4.62	±4.76

Each repetition extension peak torque (EPT) was automatically measured by the Biodex machine. EPT High represents average extension peak torque of highest three values among first six contractions, and EPT Low among last six contractions. There was a treatment by time interaction ($p = .02$) and between third and forth recovery contraction (NO-L 12.79 ± 3.08 vs. PLA 4.43 ± 2.93; $p = .03$ by 2×5 repeated ANOVA) was significantly different between treatments. *: ($p < .05$) by paired t-test, ‡: ($p < .05$) by interaction effect, and ♀: ($p < .05$) by LSD difference.

TABLE 5. Flexion Peak Torque (ft-lb), (Mean ± Standard Error)

	Exercise (1~4)								Recovery (5~10)					
Treatment	FPT 1H	FPT 2H	FPT 3H	FPT 4H	FPT 1L	FPT 2L	FPT 3L	FPT 4L	FPT 5	FPT 6	FPT 7	FPT 8	FPT 9	FPT 10
NO-L	75.69	62.06	56.39	54.35	45.48	31.76	30.87	30.11	54.29	58.50	62.64	63.57	62.29	66.21
	±3.86	±3.57	±2.97	±2.88	±3.11	±2.30	±1.89	±1.28	±4.10	±4.17	±4.96	±5.28	±5.49	±5.38
PLA	76.09	63.06	54.59	53.84	44.83	32.14	31.39	29.01	55.14	57.57	59.93	63.57	62.29	64.57
	±3.49	±3.62	±2.62	±3.12	±3.31	±2.33	±1.61	±1.47	±4.10	±3.93	±4.53	±3.91	±4.09	±4.23

Flexion peak torque (FPT) High represents average flexion peak torque of highest three values among first six contractions and FPT Low among last six contractions. Overall fatigue rate and recovery rate were significantly changed by time not by treatment.

TABLE 6. Metabolic Gases (Mean ± Standard Error)

	Treatment	Pre-resting	Post-resting	Exercise	Recovery
VO_2 (L/min)	NO-L	0.27±0.01‡	0.29±0.01	1.44±0.06	0.55±0.02
	PLA	0.27±0.01	0.27±0.01	1.45±0.06	0.54±0.02
VCO_2 (L/min)	NO-L	0.23±0.00‡	0.25±0.01	1.99±0.10‡	0.68±0.03
	PLA	0.24±0.01	0.24±0.01	2.06±0.09	0.66±0.03
RER (VCO_2/VO_2)	NO-L	0.86±0.01	0.87±0.01	1.38±0.02*	1.22±0.01
	PLA	0.89±0.01	0.88±0.02	1.42±0.02	1.22±0.02

To attain stable and reliable metabolic gas data, resting value was averaged by two to four minute values among five-minute values. Interaction of treatment by time was found for resting VO_2 and VCO_2, but there was no significant difference in resting RER. There was a significant interaction effect ($p = .018$) on VCO_2after supplementation, and VCO_2 value was lower for NO-L during exercise. There was also significant NO-L effect on exercise RER ($p = .03$). *: ($p < .05$) by paired t-test,‡: ($p < .05$)by interaction effect, and ♀: ($p < .05$) by LSD difference

TABLE 7. Work and Metabolic Gases (Mean ± Standard Error)

	Treatment	1st set	2nd set	3rd set	4th set
Work (ft-lb)	NO-L	4027.50 ±129.29	2800.50 ±100.55	2404.50 ±88.62	2279.29 ±93.67
	PLA	4007.43 ±116.98	2829.36 ±124.07	2363.14 ±99.95	2253.29 ±92.15
VO_2 (L/min)	NO-L	0.96±0.03	1.55±0.08	1.60±0.07	1.64±0.08
	PLA	0.96±0.04	1.60±0.06	1.61±0.07	1.64±0.08
VCO_2 (L/min)	NO-L	1.23±0.08	2.15±0.13	2.33±0.13	2.26±0.11
	PLA‡	1.22±0.07	2.33±0.11♀♀	2.39±0.10	2.28±0.11
RER (VCO_2/VO_2)	NO-L*	1.28±0.06	1.39±0.03	1.46±0.03	1.37±0.02
	PLA	1.27±0.05	1.46±0.05	1.49±0.03	1.40±0.02

Carbon dioxide production was found to be significantly higher at 2nd set of PLA and overall during exercise. RER was significantly lower during NO-L over the entire exercise. *: ($p < .05$) by treatment effect only, ‡: ($p < .05$) by interaction effect, and ♀: ($p < .05$) and♀♀: ($p < .01$) by LSD difference

TABLE 8. Work and VO_2 for Each Exercise Set (Mean ± Standard Error)

	Treatment	1st set	2nd set	3rd set	4th set
Work (ft-lb)	NO-L	4027.50 ±129.29	2800.50 ±100.55	2404.50 ±88.62	2279.29 ±93.67
	PLA	4007.43 ±116.98	2829.36 ±124.07	2363.14 ±99.95	2253.29 ±92.15
VO_2 (L/min)	NO-L	0.96 ±0.03	1.55 ±0.08	1.60 ±0.07	1.64 ±0.08
	PLA	0.96 ±0.04	1.60 ±0.06	1.61 ±0.07	1.64 ±0.08

Work rate was decreased over time and the difference was largest between the first and second sets. There was significant ($p = .01$) negative correlation between work and oxygen consumption for PLA($r = -.560$) and NO-L($r = -.546$)

TABLE 9. Blood Lactate and Glucose (Mean ± Standard Error)

	Treatment	Pre-resting	Post-resting	Exercise	Recovery
Lactate (mM)	NO-L	1.11 ±0.11	0.85 ±0.06	5.75 ±0.57	7.14 ±0.58
	PLA	1.22 ±0.13	0.89 ±0.05	5.94 ±0.53	6.84 ±0.57
Glucose (mM)	NO-L	4.46 ±0.07	4.55 ±0.08	4.82 ±0.10	4.86 ±0.12
	PLA	4.38 ±0.08	4.45 ±0.10	4.73 ±0.10	4.65 ±0.12

There was no change of resting blood lactate and glucose from before and after the treatment. Blood lactate steeply increased during exercise and during the recovery the concentration continued to increase. Blood glucose increased after exercise, but there was no treatment, time, or treatment by time interaction effect found.

TABLE 10. Total Work Efficiency (Mean ± Standard Error)

	NO-L	PLA
Total Work Efficiency (ft-lb/L/min)	2578.66* ± 132.24	2497.47 ± 134.56

Total work efficiency was calculated based on the sum of work done from first to the fourth set and sum of net oxygen consumption during the exercise. The result showed higher total work efficiency for NO-L, but it only approached statistical significance *:($p = .05$) by one tail paired t-test.

TABLE 11. Mean Arterial Pressure (MAP, Mean ± Standard Error)

	Treatment	Pre-resting	Post-resting	Exercise	Recovery
MAP (mmHg)	NO-L	71.86 ±2.26	78.50 ±3.04	84.86 ±3.48	74.07 ±2.19
	PLA	70.79 ±3.05	76.86 ±3.48	87.36 ±3.13	76.36 ±3.33
ΔMAP (mmHg)	NO-L			6.36 ±2.31	-4.43 ±2.12
	PLA			10.50 ±2.33	-0.50 ±3.16

Mean arterial pressure was calculated by the formula: diastolic pressure + 1/3(systolic pressure – diastolic pressure).
$\Delta MAP_{ex\text{-}postresting} = MAP_{exercise} – MAP_{post\text{-}restng}$ & $\Delta MAP_{rec\text{-}postresting} = MAP_{recovery} – MAP_{post\text{-}restng}$

Figures

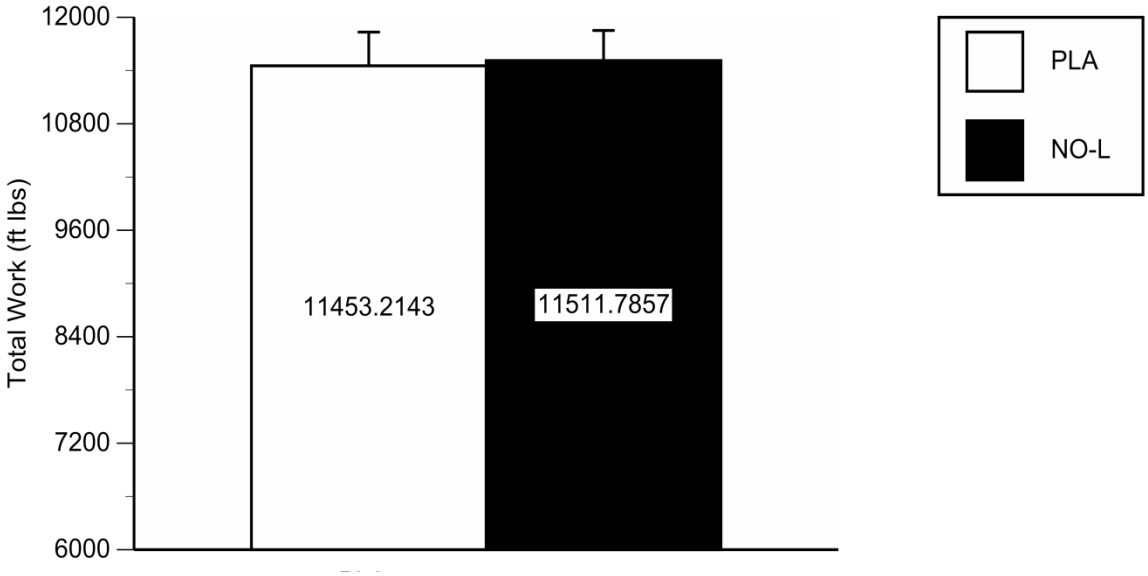


FIGURE1. Total work (TW) completed during knee extension and flexion exercise sets. There was no significant different between treatments. Bars represents means ± standard errors.

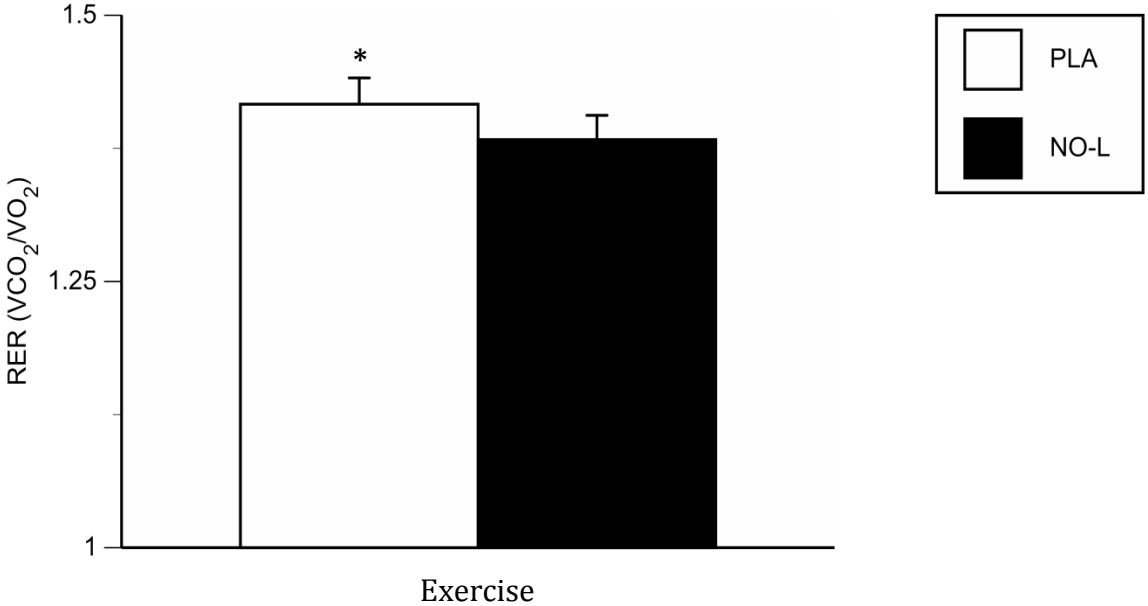


FIGURE 2. Respiratory exchange ratio (RER) during knee extension and flexion exercise sets. RER was significantly higher (p = .03) for PLA. Bars represent means ± standard errors.

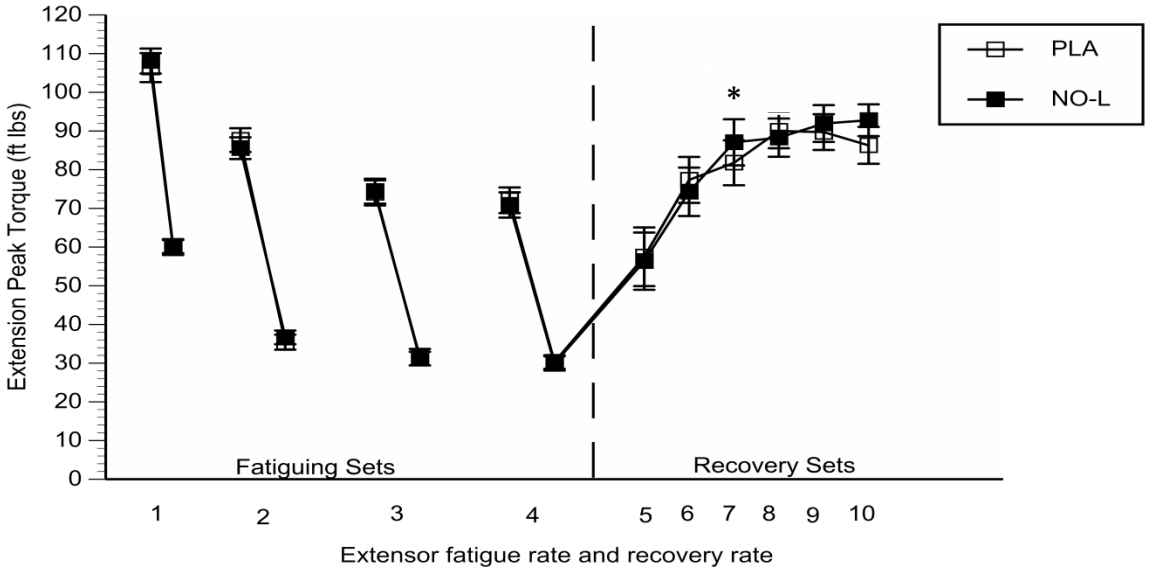


FIGURE 3. Extension peak torque during knee extension/flexion exercise and recovery. No significant treatment effect or treatment-by-time interaction was found during exercise sets, but there was a significant treatment-by-time interaction ($p = .02$) and between treatments at the 3rd recovery contraction ($p = .03$). Bars represent means ± standard errors.

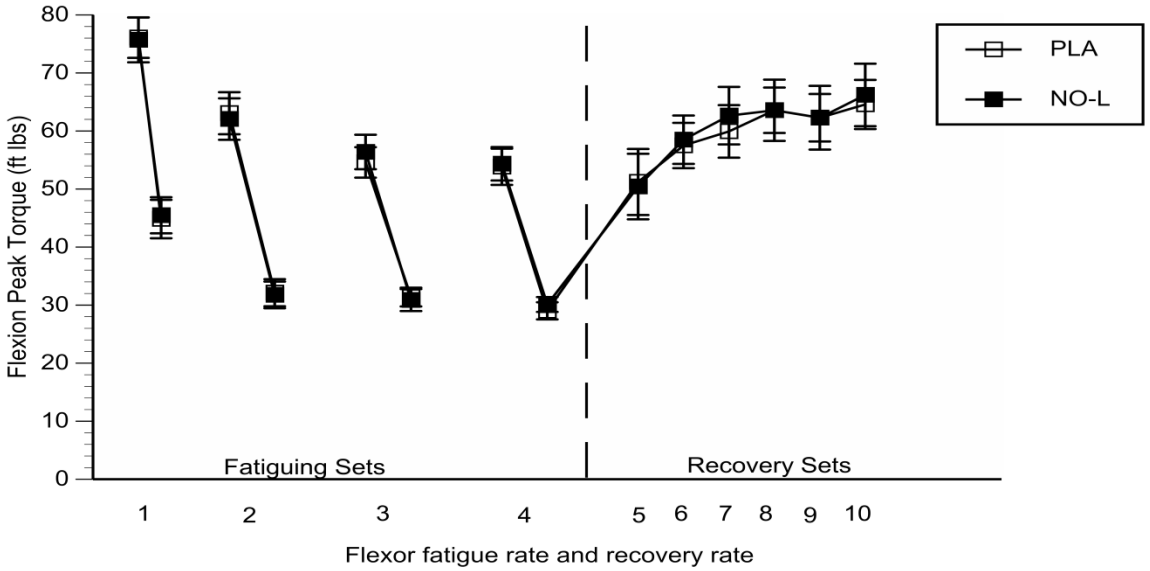


FIGURE 4. Flexion peak torque during knee extension/flexion exercise and recovery. NO significant treatment effect or treatment-by-time interaction was found. Bars represents means ± standard errors.

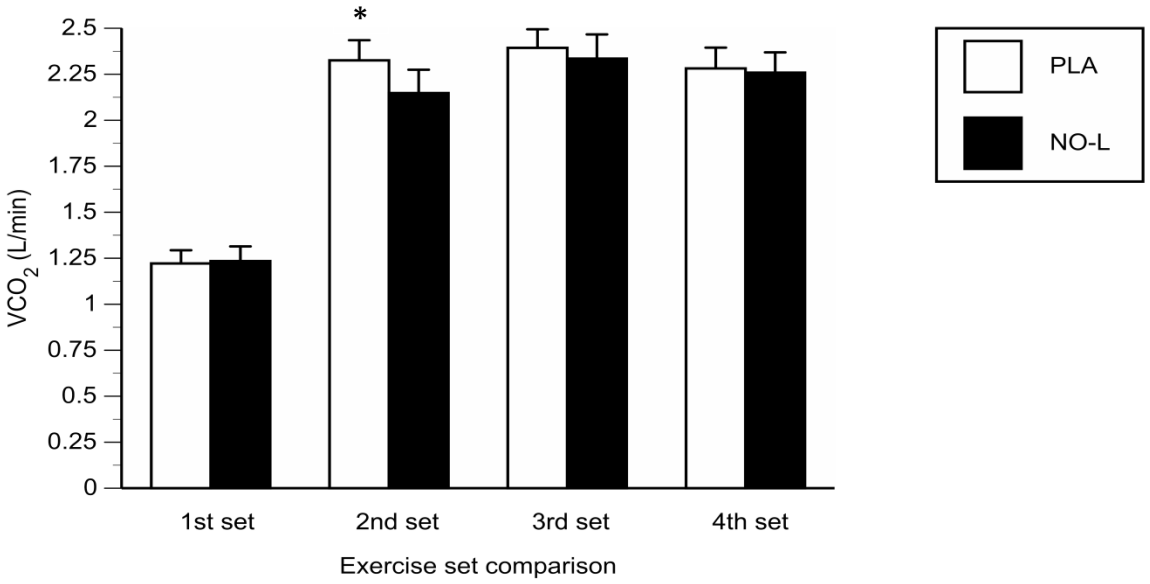


FIGURE 5. Carbon dioxide production (VCO_2) during each knee extension/flexion exercise set for each treatment. There was a significant treatment-by-time interaction (p = .01) and a significant difference between treatments for the 2nd contraction set (p = .01). Bars represent mean ± standard errors.

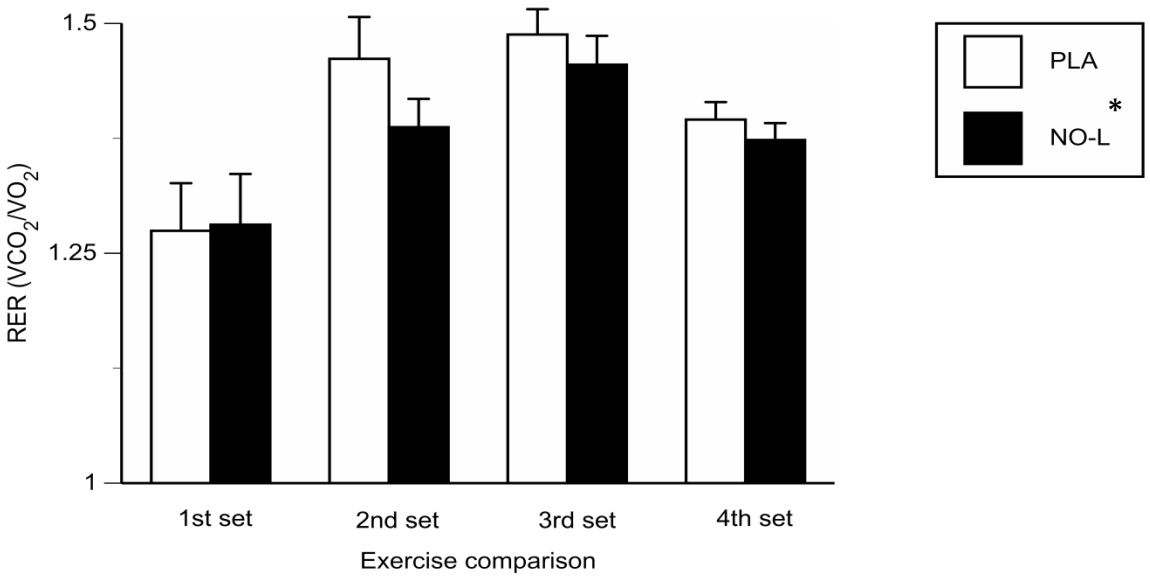


FIGURE 6. Respiratory exchange ratio (RER) during ach knee extension/flexion exercise set for each treatment. There was significant treatment effect with RER significantly lower for NO-L (p = .05). Bars represent mean ± standard errors.

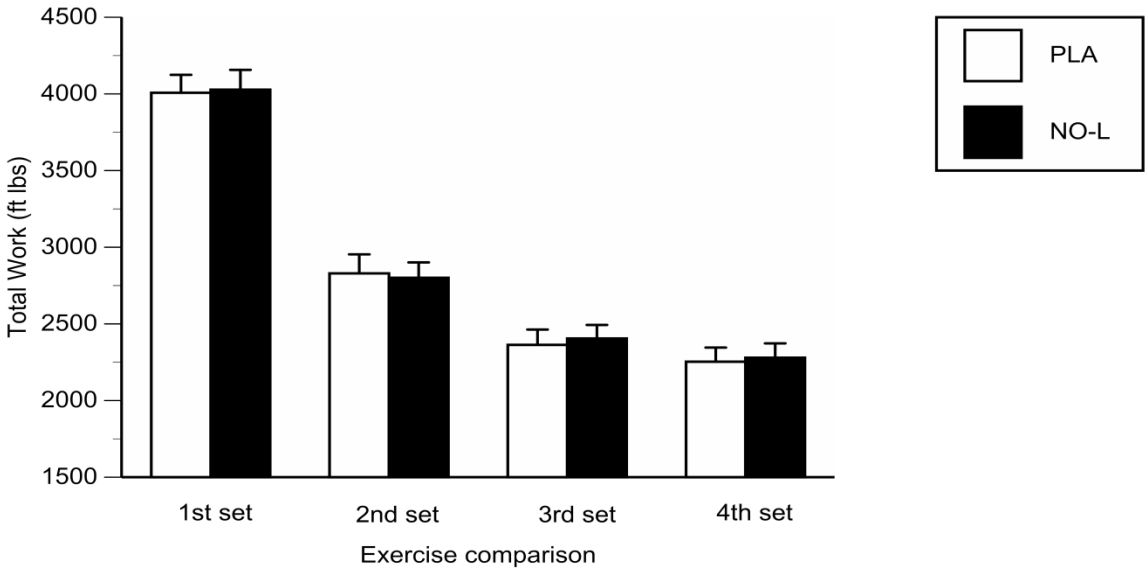


FIGURE 7. Work completed for each set of knee extension/flexion exercise. No significant differences were found between treatments. Bars represent mean ± standard errors.

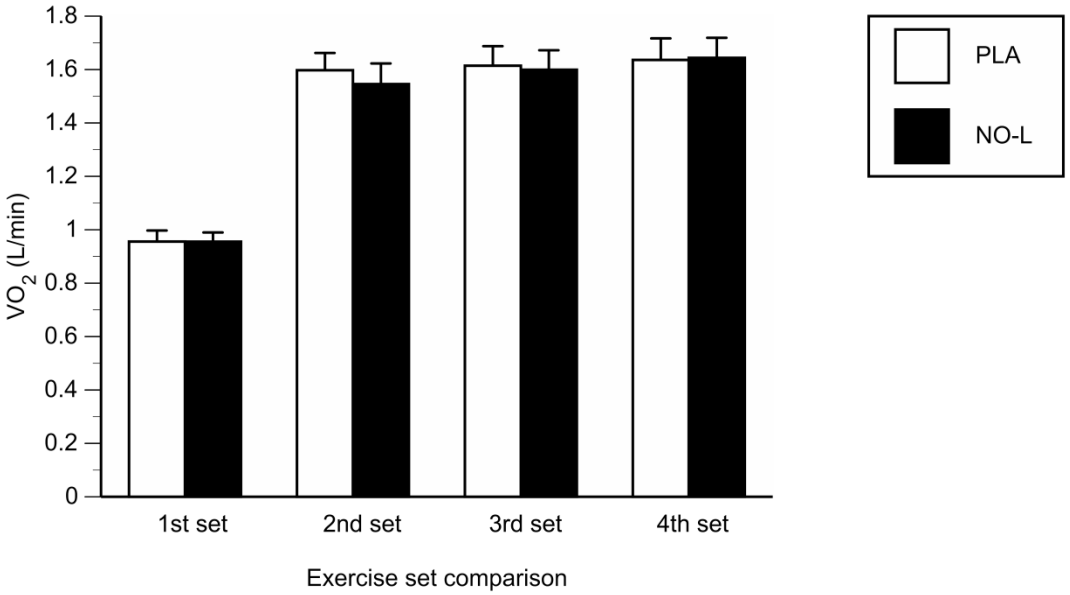


FIGURE 8. Oxygen consumption (VO_2) for each set of knee extension/flexion exercise. Bars represent mean ± standard errors.

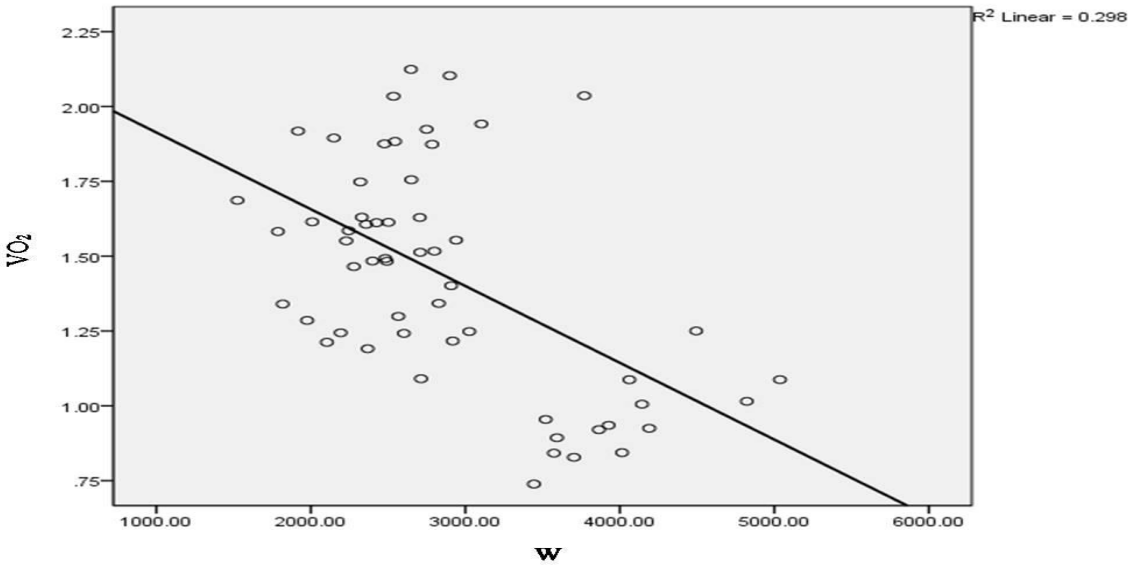


FIGURE 8.1. Correlation between oxygen consumption (VO_2) and work (W) during knee extension/flexion exercise for the NO-L treatment (r = -.546 and p < .01).

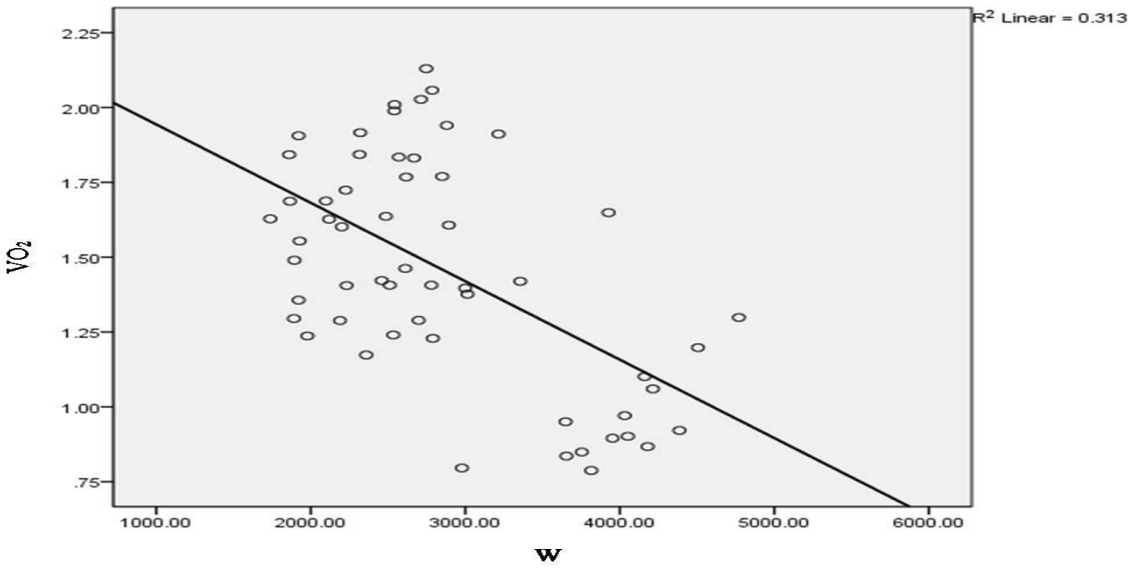


FIGURE 8.2. Correlation between oxygen consumption (VO_2) and work (W) during knee extension/flexion exercise for the PLA treatment (r = -.560 and p < .01).

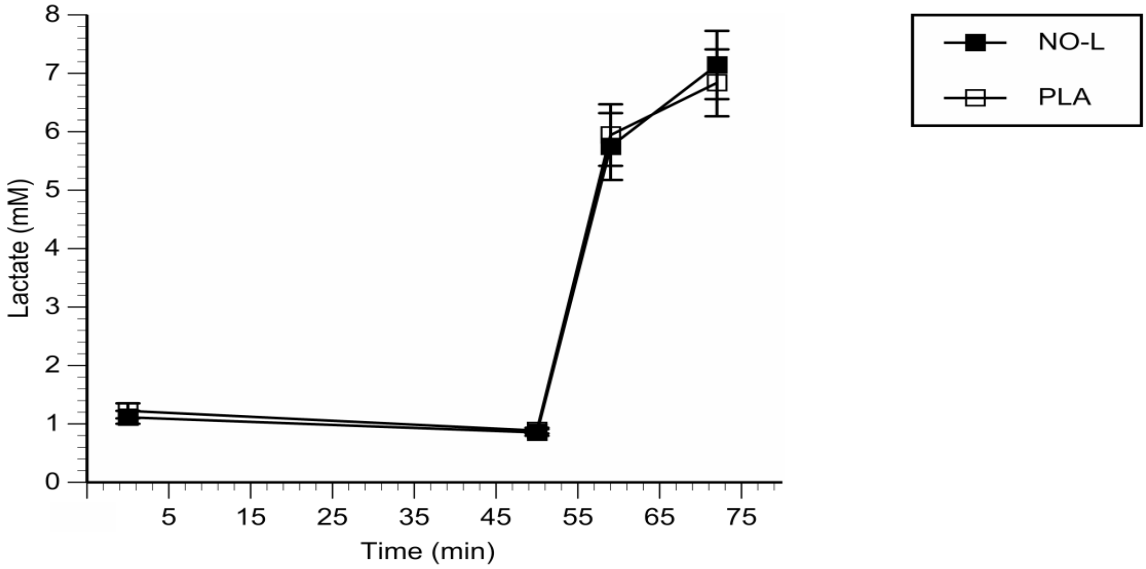


FIGURE 9. Blood lactate during experimental trials. No significant treatment effect or effect of treatment-by-time interaction was found. Bars represent mean ± standard errors.

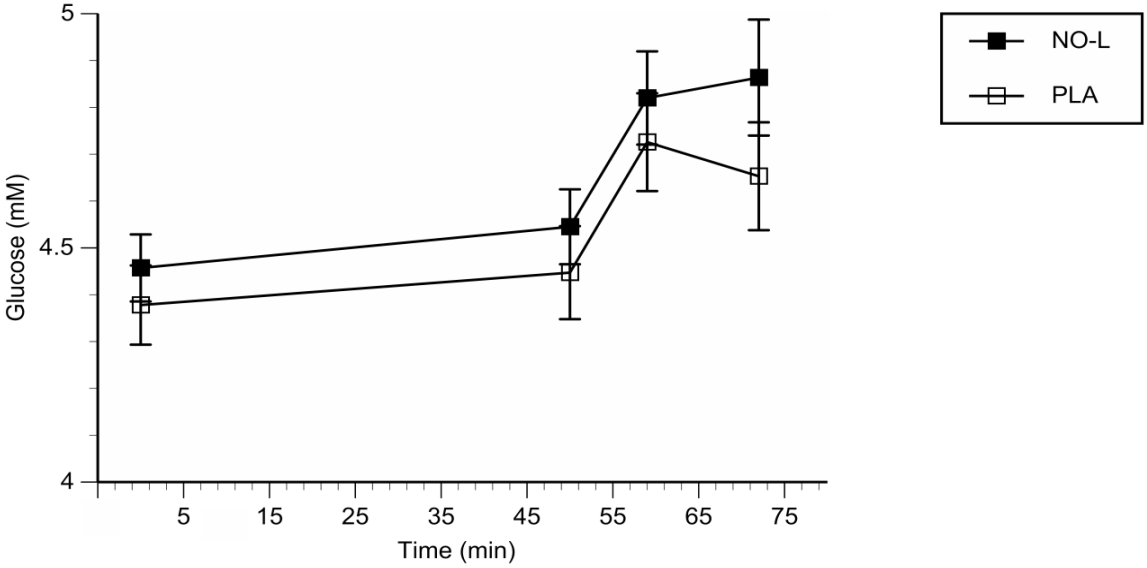


FIGURE10. Blood glucose during experimental trials. No significant treatment effect or effect of treatment-by-time interaction was found. Bars represent mean ± standard errors.

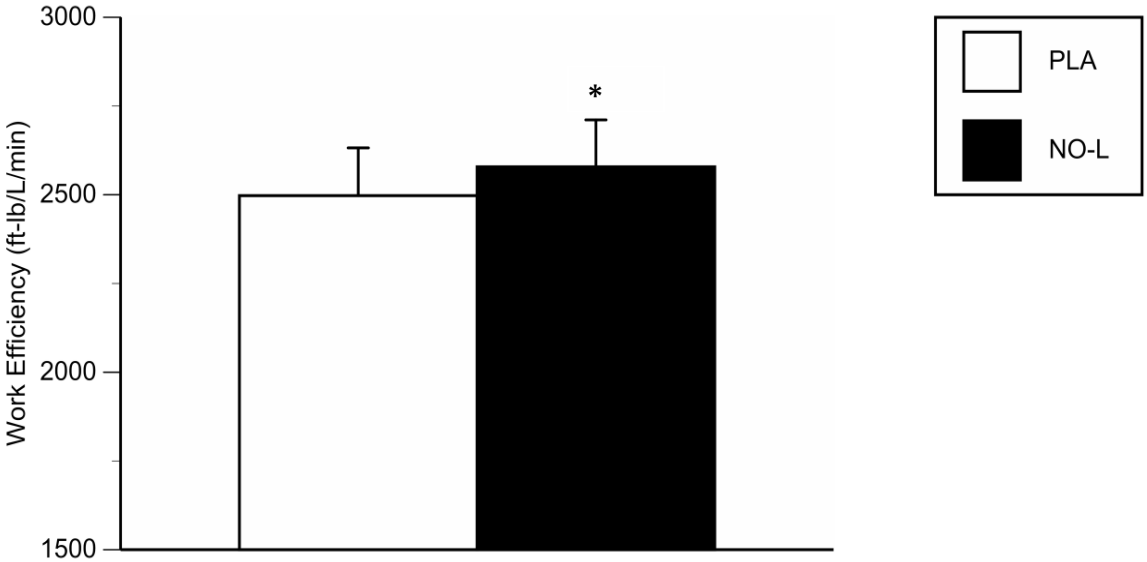


FIGURE 11. Total Work efficiency comparison during exercise. Bars represent Mean ± Standard error. No significant treatment effect was found (p = .05)

APPENDICES

Appendix A. Raw Data

A1. Subject Basic Information

Subject	Age (yr)	Height (m)	Weight (kg)	BMI (kg/m^2)	VO_{2max} (L/min)	VO_{2max} (ml/kg/min)	HR_{max} (bpm)	MAP (mmHg)	VT (L/min)	VT (RER)	VT ($\%VO_{2max}$)	VT (watt)
BLA	21	1.70	63.4	22.1	3.11	49.10	183	83.3	1.79	1.05	57.49	145
BLB	26	1.76	77.0	24.9	3.49	45.30	199	86.7	2.27	1.03	65.09	175
JRB	28	1.81	68.8	21.1	4.20	61.10	200	86.7	2.89	1.03	68.80	220
BMK	26	1.75	71.3	23.4	3.20	45.10	177	92.3	2.21	1.08	69.07	175
TLB	23	1.66	65.8	23.9	2.94	45.20	180	84.3	1.82	1.05	61.77	145
JMY	19	1.73	68.3	22.8	3.28	48.00	201	91.0	2.07	1.05	63.09	160
CWC	23	1.68	73.0	25.9	3.28	44.90	202	94.7	2.29	1.07	69.88	190
OKA	22	1.75	74.5	24.3	3.16	42.70	183	87.3	1.61	1.00	50.92	100
ERC	22	1.70	65.4	22.6	3.19	49.10	189	86.0	1.87	1.04	58.63	145
SYK	24	1.84	90.0	26.6	3.83	42.50	196	94.3	2.06	1.06	53.81	145
NLK	21	1.83	67.6	20.1	3.89	57.60	192	95.3	1.88	1.05	48.31	160
LFA	21	1.72	79.0	26.9	3.62	45.80	160	84.0	2.87	1.02	79.22	220
BTA	21	1.90	88.1	24.4	3.87	43.90	190	99.3	2.09	1.05	53.95	145
SMV	21	1.73	68.8	23.0	3.24	47.10	183	97.0	2.44	0.99	75.17	190
MEAN	22.7	1.75	72.9	23.7	3.5	47.7	188.2	90.2	2.2	1.0	62.4	165.3
SE	0.7	0.02	2.2	0.5	0.1	1.44	3.1	1.4	0.0	0.0	3.2	8.7

A2.Isokinetic Performances: Work (ft-lb, max GET uncorrected data)

	NO-L					PLA				
Subject	W 1st set	W 2nd set	W 3rd set	W 4th set	TW total	W 1st set	W 2nd set	W 3rd set	W 4th set	TW total
BLA	3573	2708	2425	2320	11026	3649	2894	2617	2320	11480
BLB	3928	2918	2602	2566	12014	4180	2790	2534	2359	11863
JRB	3593	3026	2828	2908	12355	3654	3014	2999	2780	12447
BMK	3864	2650	2149	1917	10580	4032	2540	1860	1921	10353
TLB	4061	2482	1787	1525	9855	4214	2486	1928	1738	10366
JMY	3703	2104	1818	1976	9601	3754	2232	1921	1891	9798
CWC	4190	2705	2358	2330	11583	4052	2850	2315	2226	11443
OKA	3444	2713	2368	2193	10718	3815	2698	2189	1978	10680
ERC	3520	2502	2244	2009	10275	2978	2118	1894	1865	8855
SYK	5036	2940	2492	2278	12746	4386	3355	2459	2511	12711
NLK	4494	3104	2749	2476	12823	4506	3215	2668	2570	12959
LFA	4143	2785	2545	2535	12008	4160	2880	2713	2542	12295
BTA	4822	3770	2899	2648	14139	4771	3927	2786	2747	14231
SMV	4014	2800	2399	2229	11442	3953	2612	2201	2098	10864

A3. Isokinetic Performances: Extension Peak Torque (ft-lb)

	NO-L													
Subject	EPT1H	EPT2H	EPT3H	EPT4H	EPT1L	EPT2L	EPT3L	EPT4L	EPT5	EPT6	EPT7	EPT8	EPT9	EPT10
BLA	92.7	77.7	75.0	79.7	55.0	37.3	35.0	30.3	60.0	99.0	90.0	96.0	98.0	94.0
BLB	97.7	83.0	75.3	70.7	61.3	37.3	30.0	33.0	74.0	77.0	93.0	84.0	91.0	83.0
JRB	107.0	98.7	91.3	92.7	65.3	49.7	45.0	48.7	112.0	103.0	106.0	104.0	106.0	103.0
BMK	102.7	72.0	54.0	49.3	58.0	30.7	25.7	20.3	12.0	15.0	36.0	53.0	63.0	74.0
TLB	123.3	85.7	62.0	59.0	63.0	31.3	25.3	22.3	48.0	53.0	82.0	78.0	85.0	99.0
JMY	104.0	77.3	64.0	70.3	52.0	25.3	21.7	24.3	65.0	73.0	66.0	86.0	77.0	74.0
CWC	104.0	90.3	72.7	72.0	62.7	37.3	36.7	33.0	51.0	83.0	104.0	103.0	108.0	111.0
OKA	114.0	86.0	88.7	78.3	47.0	45.7	39.7	34.0	62.0	70.0	87.0	69.0	89.0	76.0
ERC	85.3	69.7	69.3	66.3	59.0	29.7	26.0	27.3	62.0	62.0	68.0	73.0	73.0	70.0
SYK	121.7	88.7	65.7	53.3	71.7	34.0	24.3	29.0	45.0	70.0	93.0	94.0	78.0	97.0
NLK	103.0	74.0	64.3	61.0	55.7	32.7	31.0	32.0	54.0	69.0	74.0	82.0	89.0	94.0
LFA	126.7	98.0	92.7	86.0	62.3	39.7	33.0	32.0	87.0	111.0	130.0	128.0	132.0	120.0
BTA	119.3	103.0	84.0	75.7	66.7	40.3	29.0	26.0	65.0	82.0	104.0	104.0	110.0	108.0
SMV	111.7	93.7	83.3	78.0	62.0	42.3	34.3	26.7	52.0	73.0	86.0	82.0	88.0	96.0

	PLA													
Subject	EPT1H	EPT2H	EPT3H	EPT4H	EPT1L	EPT2L	EPT3L	EPT4L	EPT5	EPT6	EPT7	EPT8	EPT9	EPT10
BLA	95.3	81.0	79.0	76.7	65.0	42.7	37.7	31.3	63.0	94.0	90.0	85.0	95.0	88.0
BLB	108.0	86.0	74.7	72.3	64.7	30.0	35.0	32.7	71.0	96.0	94.0	104.0	93.0	96.0
JRB	112.0	98.3	95.0	94.7	58.0	45.3	50.0	48.7	114.0	113.0	116.0	112.0	111.0	108.0
BMK	96.0	70.0	51.3	44.7	51.7	26.0	19.7	21.3	13.0	37.0	51.0	72.0	70.0	78.0
TLB	133.3	83.3	68.0	67.0	61.7	29.0	24.3	24.0	55.0	54.0	64.0	80.0	74.0	91.0
JMY	100.3	80.3	68.3	66.0	49.0	25.0	22.3	26.0	52.0	63.0	64.0	70.0	69.0	62.0
CWC	107.3	92.3	72.0	69.7	67.3	44.3	36.3	34.0	63.0	83.0	63.0	88.0	90.0	69.0
OKA	115.7	92.0	78.7	72.3	55.0	43.0	26.3	25.7	70.0	74.0	83.0	92.0	96.0	84.0
ERC	84.7	70.7	66.0	71.3	45.0	31.3	27.0	26.3	76.0	71.0	75.0	75.0	75.0	70.0
SYK	84.0	90.3	63.0	61.3	67.3	40.3	31.7	30.0	47.0	61.0	67.0	72.0	72.0	62.0
NLK	103.3	79.0	68.3	65.3	56.0	32.7	26.7	27.3	60.0	73.0	65.0	86.0	91.0	81.0
LFA	124.7	108.3	98.7	92.0	66.7	31.3	39.0	34.0	88.0	118.0	126.0	127.0	131.0	121.0
BTA	117.3	105.7	78.0	81.0	69.7	43.0	34.0	31.7	47.0	79.0	98.0	101.0	95.0	108.0
SMV	107.3	90.0	75.0	75.0	63.0	32.0	31.7	31.3	49.0	67.0	89.0	96.0	94.0	90.0

A4. Isokinetic Performances: Flexion Peak Torque (ft-lb)

	NO-L													
Subject	FPT1H	FPT2H	FPT3H	FPT4H	FPT1L	FPT2L	FPT3L	FPT4L	FPT5	FPT6	FPT7	FPT8	FPT9	FPT10
BLA	60.7	50.7	55.0	49.7	37.3	32.0	32.7	29.0	52.0	60.0	65.0	61.0	70.0	67.0
BLB	69.7	66.7	64.7	62.0	44.3	37.7	39.0	36.3	53.0	50.0	59.0	52.0	62.0	52.0
JRB	60.3	55.3	53.7	55.3	45.7	26.7	26.0	31.0	61.0	53.0	58.0	57.0	48.0	53.0
BMK	73.0	60.7	51.3	47.3	41.7	27.0	28.3	32.0	29.0	29.0	33.0	36.0	34.0	48.0
TLB	76.0	49.7	41.7	38.3	41.0	28.3	22.3	25.3	41.0	46.0	54.0	55.0	53.0	58.0
JMY	72.7	52.3	43.7	44.0	40.7	23.3	26.3	27.3	54.0	58.0	62.0	61.0	59.0	64.0
CWC	75.3	55.7	52.0	51.0	43.3	26.0	26.3	27.3	51.0	66.0	72.0	76.0	75.0	75.0
OKA	68.7	54.7	52.3	46.7	29.3	26.3	28.0	25.3	28.0	40.0	25.0	26.0	18.0	25.0
ERC	64.0	55.3	52.0	50.3	43.0	27.0	24.0	24.0	57.0	60.0	57.0	61.0	61.0	59.0
SYK	108.0	85.0	72.0	68.0	52.7	34.7	41.0	33.3	82.0	86.0	92.0	102.0	98.0	104.0
NLK	89.0	70.7	64.3	67.0	63.3	48.3	42.0	38.7	63.0	73.0	72.0	77.0	78.0	81.0
LFA	73.0	60.0	56.0	58.3	38.0	25.3	24.0	25.3	61.0	63.0	78.0	78.0	66.0	80.0
BTA	102.3	95.3	82.7	76.7	75.7	51.3	41.3	37.0	78.0	83.0	90.0	88.0	88.0	95.0
SMV	67.0	56.7	48.0	46.3	40.7	30.7	31.0	29.7	48.0	52.0	60.0	60.0	62.0	66.0

	PLA													
Subject	FPT1H	FPT2H	FPT3H	FPT4H	FPT1L	FPT2L	FPT3L	FPT4L	FPT5	FPT6	FPT7	FPT8	FPT9	FPT10
BLA	67.7	55.3	49.0	52.3	40.0	36.0	35.3	27.0	53.0	61.0	66.0	58.0	54.0	57.0
BLB	76.0	64.0	60.7	60.7	45.0	33.3	38.7	34.3	47.0	49.0	53.0	55.0	59.0	59.0
JRB	61.7	57.0	58.7	51.7	41.7	33.3	36.0	27.0	72.0	64.0	61.0	57.0	63.0	60.0
BMK	70.7	65.0	46.0	46.3	39.0	25.7	26.3	36.0	28.0	34.0	40.0	45.0	32.0	40.0
TLB	74.0	55.3	45.7	41.0	45.3	29.7	27.7	26.3	46.0	49.0	52.0	58.0	55.0	61.0
JMY	74.0	53.0	46.7	42.7	35.7	23.3	23.7	22.7	49.0	53.0	54.0	56.0	58.0	58.0
CWC	73.7	59.0	47.0	49.0	44.0	26.3	29.7	26.0	57.0	61.0	64.0	68.0	64.0	67.0
OKA	73.0	56.7	59.3	50.3	33.3	25.7	24.0	25.0	33.0	41.0	27.0	55.0	60.0	52.0
ERC	62.7	44.3	39.7	39.3	39.0	24.3	23.0	25.3	55.0	48.0	52.0	50.0	48.0	48.0
SYK	103.3	83.7	68.7	71.0	52.0	41.3	37.7	41.0	82.0	93.0	99.0	97.0	93.0	98.0
NLK	89.7	76.3	67.0	66.7	66.3	44.3	38.3	32.3	51.0	65.0	65.0	64.0	68.0	82.0
LFA	74.0	63.7	58.7	58.7	27.7	22.3	31.0	22.3	67.0	56.0	70.0	78.0	70.0	75.0
BTA	101.0	95.3	69.7	78.0	74.3	51.7	39.3	33.7	77.0	76.0	78.0	88.0	89.0	87.0
SMV	63.7	54.3	47.3	46.0	44.3	32.7	28.7	27.3	53.0	56.0	58.0	61.0	59.0	60.0

A5. Isokinetic Performances: Time to Peak Torque (Second, Average values)

	NO-L								PLA							
	1st		2nd		3rd		4th		1st		2nd		3rd		4th	
Subject	EX	FL	EX	FL	EX	FL	EX	FL	EX	FL	EX	FL	EX	FL	EX	FL
BLA	0.23	0.25	0.23	0.22	0.22	0.2	0.23	0.18	0.23	0.23	0.21	0.18	0.24	0.17	0.23	0.17
BLB	0.23	0.2	0.24	0.12	0.25	0.13	0.24	0.14	0.24	0.22	0.25	0.17	0.25	0.14	0.25	0.13
JRB	0.21	0.24	0.23	0.24	0.24	0.23	0.24	0.2	0.2	0.26	0.22	0.24	0.22	0.23	0.2	0.2
BMK	0.21	0.21	0.2	0.26	0.21	0.27	0.21	0.25	0.23	0.26	0.21	0.28	0.23	0.27	0.22	0.26
TLB	0.21	0.21	0.19	0.2	0.17	0.15	0.15	0.18	0.19	0.18	0.2	0.2	0.18	0.16	0.16	0.18
JMY	0.22	0.27	0.22	0.25	0.21	0.26	0.23	0.23	0.23	0.26	0.23	0.26	0.23	0.25	0.24	0.26
CWC	0.21	0.22	0.21	0.19	0.19	0.14	0.17	0.11	0.22	0.25	0.24	0.21	0.23	0.2	0.23	0.24
OKA	0.2	0.27	0.18	0.22	0.19	0.22	0.17	0.19	0.18	0.2	0.15	0.2	0.16	0.22	0.16	0.23
ERC	0.2	0.2	0.21	0.22	0.21	0.2	0.22	0.19	0.21	0.23	0.24	0.23	0.2	0.2	0.18	0.2
SYK	0.2	0.2	0.14	0.2	0.15	0.16	0.14	0.23	0.2	0.24	0.23	0.25	0.21	0.25	0.22	0.24
NLK	0.2	0.2	0.14	0.2	0.15	0.16	0.14	0.23	0.2	0.24	0.23	0.25	0.21	0.25	0.22	0.24
LFA	0.18	0.21	0.2	0.22	0.18	0.21	0.24	0.24	0.24	0.22	0.23	0.21	0.2	0.22	0.23	0.19
BTA	0.22	0.22	0.2	0.22	0.19	0.23	0.18	0.21	0.21	0.23	0.21	0.24	0.21	0.21	0.2	0.18
SMV	0.2	0.27	0.21	0.25	0.2	0.22	0.18	0.16	0.21	0.23	0.2	0.23	0.2	0.17	0.17	0.16

A6. Metabolic Gases: VO_2 (l/min)

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	0.2457	0.2568	1.4286	0.5058	0.2664	0.2876	1.5603	0.5818
BLB	0.2757	0.2920	1.1731	0.4376	0.2626	0.2726	1.1274	0.4432
JRB	0.2886	0.2895	1.2213	0.4736	0.2762	0.2542	1.2537	0.4487
BMK	0.2894	0.3123	1.6221	0.6223	0.2760	0.2796	1.6769	0.6218
TLB	0.2549	0.2855	1.4620	0.5621	0.2983	0.2959	1.4696	0.6058
JMY	0.2686	0.2673	1.1664	0.5017	0.2287	0.2347	1.2264	0.4794
CWC	0.2498	0.2959	1.4476	0.6029	0.2426	0.2816	1.5595	0.6018
OKA	0.2430	0.2662	1.0660	0.3975	0.2926	0.2698	1.1504	0.434
ERC	0.2686	0.2655	1.4418	0.5554	0.2426	0.2094	1.3998	0.4571
SYK	0.3023	0.3205	1.3972	0.5923	0.2947	0.2742	1.2922	0.5217
NLK	0.2639	0.2591	1.7477	0.6278	0.2730	0.2915	1.6937	0.593
LFA	0.2398	0.2653	1.6991	0.607	0.2328	0.2529	1.7694	0.6276
BTA	0.3235	0.3526	1.8195	0.6936	0.3106	0.3311	1.7836	0.6729
SMV	0.3014	0.3016	1.4117	0.5515	0.2974	0.2871	1.3488	0.4916
MEAN	0.2725	0.2879	1.4360	0.5522	0.2710	0.2730	1.4508	0.5415
SE	0.0068	0.0073	0.0609	0.0217	0.0071	0.0078	0.0606	0.0218

A7. Metabolic Gases: VCO_2 (l/min)

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	0.2198	0.2523	1.9589	0.6263	0.2329	0.2506	2.2247	0.7438
BLB	0.2165	0.2253	1.6520	0.4825	0.2215	0.2197	1.6886	0.5019
JRB	0.2442	0.2556	1.5944	0.5640	0.2413	0.2136	1.5740	0.4940
BMK	0.2392	0.2883	2.2399	0.7508	0.2431	0.246	2.3176	0.7556
TLB	0.2156	0.2507	2.1004	0.7284	0.2687	0.2732	2.2017	0.7805
JMY	0.2392	0.2391	1.6055	0.6327	0.1983	0.2049	1.7285	0.6039
CWC	0.2209	0.2412	1.7782	0.7654	0.2131	0.2172	2.0424	0.7877
OKA	0.2236	0.2354	1.4464	0.4845	0.2848	0.2504	1.5928	0.5102
ERC	0.2266	0.2279	1.9404	0.6713	0.213	0.2023	1.9962	0.5280
SYK	0.2559	0.2704	2.1069	0.7246	0.2898	0.2623	2.0702	0.6681
NLK	0.2344	0.2417	2.7622	0.8163	0.2367	0.2663	2.6037	0.7309
LFA	0.2334	0.2261	2.3583	0.7388	0.2099	0.2352	2.3657	0.7706
BTA	0.2701	0.3126	2.5134	0.8684	0.2787	0.2964	2.4940	0.8347
SMV	0.2326	0.2462	1.8565	0.6451	0.2332	0.2366	1.8767	0.5882
MEAN	0.2337	0.2509	1.9943	0.6786	0.2404	0.2411	2.0550	0.6636
SE	0.0041	0.0066	0.1012	0.0312	0.0079	0.0074	0.0886	0.0328

A8. Metabolic Gases: RER

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	0.8944	0.9826	1.3712	1.2382	0.8743	0.8713	1.4259	1.2784
BLB	0.7851	0.7716	1.4082	1.1026	0.8435	0.8058	1.4978	1.1324
JRB	0.846	0.8832	1.3055	1.1907	0.8736	0.8402	1.2555	1.1011
BMK	0.8267	0.9232	1.3809	1.2065	0.8805	0.8797	1.3821	1.2153
TLB	0.8458	0.8779	1.4367	1.2958	0.9009	0.9234	1.4981	1.2885
JMY	0.8907	0.8943	1.3765	1.2611	0.8671	0.8732	1.4094	1.2597
CWC	0.8843	0.8153	1.2284	1.2697	0.8784	0.7711	1.3096	1.3088
OKA	0.9204	0.8845	1.3569	1.2189	0.9736	0.9278	1.3845	1.1756
ERC	0.8437	0.8582	1.3458	1.2087	0.8778	0.9662	1.426	1.155
SYK	0.8465	0.8436	1.5079	1.2234	0.9836	0.9564	1.6021	1.2806
NLK	0.8883	0.9327	1.5805	1.3003	0.867	0.9137	1.5373	1.2326
LFA	0.9731	0.8523	1.388	1.2171	0.9016	0.93	1.337	1.2278
BTA	0.8418	0.8864	1.3596	1.2331	0.897	0.8964	1.3811	1.2165
SMV	0.7728	0.8166	1.3184	1.1443	0.7848	0.8253	1.3846	1.1697
MEAN	0.8614	0.8730	1.3832	1.2222	0.8860	0.8843	1.4165	1.2173
SE	0.0140	0.0143	0.0229	0.0144	0.0131	0.0154	0.0247	0.0169

A9. Metabolic Gases: Each Exercise Set VO_2 (l/min)

	NO-L				PLA			
Subject	1st set	2nd set	3rd set	4th set	1st set	2nd set	3rd set	4th set
BLA	0.8419	1.5129	1.6118	1.7480	0.9504	1.6069	1.7677	1.9160
BLB	0.9349	1.2165	1.2421	1.2989	0.8673	1.2288	1.2402	1.1732
JRB	0.8933	1.2483	1.3423	1.4013	0.8356	1.3763	1.3964	1.4064
BMK	0.9203	1.7554	1.8948	1.9178	0.9707	1.9888	1.8424	1.9056
TLB	1.0872	1.4921	1.5823	1.6863	1.0601	1.6364	1.5540	1.6281
JMY	0.8280	1.2122	1.3400	1.2853	0.8491	1.4053	1.3564	1.2949
CWC	0.9250	1.6292	1.6062	1.6299	0.9015	1.7695	1.8431	1.7239
OKA	0.7386	1.0906	1.1908	1.2440	0.7875	1.2890	1.2883	1.2369
ERC	0.9546	1.6130	1.5849	1.6147	0.7958	1.6269	1.4898	1.6867
SYK	1.0874	1.5535	1.4827	1.4651	0.9212	1.4193	1.4220	1.4064
NLK	1.2500	1.9416	1.9237	1.8754	1.1979	1.9114	1.8312	1.8343
LFA	1.0054	1.8736	1.8831	2.0341	1.1011	1.9401	2.0271	2.0095
BTA	1.0150	2.0360	2.1030	2.1240	1.2984	1.6486	2.0576	2.1297
SMV	0.8952	1.4623	1.6014	1.6877	0.8436	1.5167	1.4838	1.5511

A10. Metabolic Gases: Each Exercise Set VCO_2 (l/min)

	NO-L				PLA			
Subject	1st set	2nd set	3rd set	4th set	1st set	2nd set	3rd set	4th set
BLA	1.2536	1.9821	2.1954	2.4045	1.3448	2.2589	2.5269	1.2536
BLB	1.3790	1.8180	1.7385	1.6724	1.2857	1.9916	1.8700	1.3790
JRB	1.0702	1.6216	1.8276	1.8580	1.0087	1.6642	1.8492	1.0702
BMK	1.0132	2.3548	2.9184	2.6733	1.1361	2.8055	2.7528	1.0132
TLB	1.7052	2.0481	2.3568	2.2916	1.7409	2.4423	2.3687	1.7052
JMY	0.8509	1.6223	2.0607	1.8882	0.8890	1.9985	2.1053	0.8509
CWC	0.8394	1.9511	2.1920	2.1303	0.8564	2.1917	2.5961	0.8394
OKA	0.8548	1.5643	1.6646	1.7021	0.9363	1.8903	1.8871	0.8548
ERC	1.1130	2.1663	2.2835	2.1988	1.0930	2.2255	2.3481	1.1130
SYK	1.4798	2.4676	2.3618	2.1183	1.2697	2.4699	2.4177	1.4798
NLK	1.6646	3.1470	3.3739	2.8635	1.6633	3.0760	2.9734	1.6646
LFA	1.6081	2.5265	2.6073	2.6914	1.3960	2.6426	2.7693	1.6081
BTA	1.2458	2.8701	2.9932	2.9444	1.3608	2.8648	2.8700	1.2458
SMV	1.2013	1.9247	2.1147	2.1855	1.1199	2.0355	2.1732	1.2013

A11. Metabolic Gases: Each Exercise Set RER

	NO-L				PLA			
Subject	1st set	2nd set	3rd set	4th set	1st set	2nd set	3rd set	4th set
BLA	1.489	1.3102	1.3621	1.3756	1.415	1.4058	1.4295	1.4447
BLB	1.475	1.4944	1.3997	1.2876	1.4823	1.6207	1.5078	1.3699
JRB	1.198	1.2991	1.3616	1.326	1.2071	1.2092	1.3242	1.2612
BMK	1.1009	1.3415	1.5403	1.3939	1.1703	1.4107	1.4941	1.3519
TLB	1.5685	1.3726	1.4894	1.3589	1.6422	1.4925	1.5242	1.3851
JMY	1.0276	1.3384	1.5378	1.4691	1.0469	1.4221	1.5522	1.4837
CWC	0.9075	1.1976	1.3647	1.307	0.9499	1.2386	1.4086	1.4649
OKA	1.1573	1.4343	1.3979	1.3682	1.1889	1.4665	1.4648	1.34
ERC	1.1659	1.3431	1.4408	1.3618	1.3735	1.3679	1.5762	1.3743
SYK	1.3608	1.5884	1.5929	1.4458	1.3784	1.7402	1.7002	1.51
NLK	1.3316	1.6208	1.7539	1.5268	1.3885	1.6093	1.6237	1.4732
LFA	1.5994	1.3484	1.3846	1.3231	1.2679	1.3621	1.3661	1.3212
BTA	1.2123	1.4148	1.4255	1.3858	1.0041	1.7739	1.3948	1.3514
SMV	1.3419	1.3162	1.3205	1.2949	1.3275	1.342	1.4646	1.4043

A12. Correlation between Each Exercise Set VO_2 (l/min) and WD (ft-lb)

	NO-L							
Subject	$1^{st}VO_2$	$1^{st}W$	$2^{nd}VO_2$	$2^{nd}W$	$3^{rd}VO_2$	$3^{rd}W$	$4^{th}VO_2$	$4^{th}W$
BLA	0.8419	3573	1.5129	2708	1.6118	2425	1.7480	2320
BLB	0.9349	3928	1.2165	2918	1.2421	2602	1.2989	2566
JRB	0.8933	3593	1.2483	3026	1.3423	2828	1.4013	2908
BMK	0.9203	3864	1.7554	2650	1.8948	2149	1.9178	1917
TLB	1.0872	4061	1.4921	2482	1.5823	1787	1.6863	1525
JMY	0.8280	3703	1.2122	2104	1.3400	1818	1.2853	1976
CWC	0.9250	4190	1.6292	2705	1.6062	2358	1.6299	2330
OKA	0.7386	3444	1.0906	2713	1.1908	2368	1.2440	2193
ERC	0.9546	3520	1.6130	2502	1.5849	2244	1.6147	2009
SYK	1.0874	5036	1.5535	2940	1.4827	2492	1.4651	2278
NLK	1.2500	4494	1.9416	3104	1.9237	2749	1.8754	2476
LFA	1.0054	4143	1.8736	2785	1.8831	2545	2.0341	2535
BTA	1.0150	4822	2.0360	3770	2.1030	2899	2.1240	2648
SMV	0.8952	4014	1.4623	2800	1.6014	2399	1.6877	2229

	PLA							
Subject	1st VO_2	1stW	2ndVO_2	2ndW	3rd VO_2	3rdW	4thVO_2	4thW
BLA	0.9504	3649	1.6069	2894	1.7677	2617	1.9160	2320
BLB	0.8673	4180	1.2288	2790	1.2402	2534	1.1732	2359
JRB	0.8356	3654	1.3763	3014	1.3964	2999	1.4064	2780
BMK	0.9707	4032	1.9888	2540	1.8424	1860	1.9056	1921
TLB	1.0601	4214	1.6364	2486	1.5540	1928	1.6281	1738
JMY	0.8491	3754	1.4053	2232	1.3564	1921	1.2949	1891
CWC	0.9015	4052	1.7695	2850	1.8431	2315	1.7239	2226
OKA	0.7875	3815	1.2890	2698	1.2883	2189	1.2369	1978
ERC	0.7958	2978	1.6269	2118	1.4898	1894	1.6867	1865
SYK	0.9212	4386	1.4193	3355	1.4220	2459	1.4064	2511
NLK	1.1979	4506	1.9114	3215	1.8312	2668	1.8343	2570
LFA	1.1011	4160	1.9401	2880	2.0271	2713	2.0095	2542
BTA	1.2984	4771	1.6486	3927	2.0576	2786	2.1297	2747
SMV	0.8436	3953	1.5167	2612	1.4838	2201	1.5511	2098

A13. Work Efficiency (ft-lb/l/min)

	NO-L			PLA		
Subject	Net VO_2 (l/4min)	TW (ft-lb/4min)	WE (ft-lb/l/min)	Net VO_2 (l/4min)	TW (ft-lb/4min)	WE (ft-lb/l/min)
BLA	4.6872	11026	2352.36	5.0904	11480	2255.23
BLB	3.5244	12014	3408.81	3.4192	11863	3469.53
JRB	3.7272	12355	3314.82	3.998	12447	3113.31
BMK	5.2392	10580	2019.39	5.5892	10353	1852.32
TLB	4.706	9855	2094.14	4.6952	10366	2207.79
JMY	3.596	9601	2669.91	3.9672	9798	2469.75
CWC	4.6068	11583	2514.33	5.1116	11443	2238.63
OKA	3.1992	10718	3350.21	3.5224	10680	3032.02
ERC	4.7048	10275	2183.94	4.7616	8855	1859.67
SYK	4.3068	12746	2959.51	4.072	12711	3121.56
NLK	5.954	12823	2153.68	5.6088	12959	2310.48
LFA	5.7352	12008	2093.74	6.0664	12295	2026.74
BTA	5.8676	14139	2409.67	5.81	14231	2449.40
SMV	4.4404	11442	2576.79	4.2468	10864	2558.16

Net VO_2 = Exercise VO_2 – Post-resting VO_2, TWD =Sum of work done from 1^{st} set to 4^{th} set

A14. Blood Lactate (mM)

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	0.87	0.92	7.30	6.50	0.82	0.72	7.50	7.98
BLB	0.85	0.75	4.02	4.78	0.85	0.82	4.96	4.99
JRB	1.10	0.69	4.21	3.83	1.13	0.69	2.84	3.82
BMK	0.96	0.95	4.88	9.86	0.87	0.80	4.60	8.19
TLB	1.03	1.07	9.40	9.99	1.03	1.07	9.40	9.99
JMY	1.75	1.43	8.59	6.81	2.17	1.05	8.45	8.76
CWC	1.52	0.89	3.86	7.08	1.36	0.99	6.12	7.72
OKA	0.68	0.64	3.66	4.46	1.36	0.83	4.18	3.52
ERC	0.84	0.77	4.71	7.65	0.65	0.77	6.51	5.22
SYK	1.89	0.93	3.83	6.99	1.50	1.35	7.19	7.96
NLK	0.82	0.81	9.67	11.76	0.80	0.81	7.76	9.88
LFA	0.71	0.83	6.23	6.42	1.02	1.00	5.91	5.28
BTA	1.68	0.66	5.44	7.12	2.21	0.75	4.46	7.24
SMV	0.88	0.61	4.65	6.73	1.36	0.75	3.32	5.16

A15. Blood Glucose (mM)

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	4.01	4.17	4.51	4.30	4.58	4.60	4.98	4.51
BLB	4.31	4.49	4.77	4.76	4.58	4.46	4.74	4.59
JRB	4.09	4.35	4.53	4.30	3.96	4.05	4.13	4.14
BMK	4.36	4.62	4.83	5.32	4.51	4.74	4.9	5.02
TLB	4.73	4.66	5.46	5.56	4.41	4.73	5.72	5.74
JMY	4.36	4.61	4.5	4.74	4.15	4.29	4.55	4.67
CWC	4.39	4.51	4.87	5.05	4.29	4.37	4.7	4.89
OKA	4.42	4.48	4.61	4.66	3.97	4.23	4.32	4.07
ERC	4.89	5.11	5.43	5.66	4.77	4.77	4.87	4.87
SYK	4.4	4.18	4.59	4.66	4.54	4.69	4.74	4.65
NLK	4.45	4.51	4.59	4.88	4.27	4.29	4.5	4.53
LFA	4.69	4.3	4.69	4.6	3.79	3.47	4.25	4.08
BTA	4.93	5.20	5.53	5.35	4.80	4.79	4.93	4.79
SMV	4.37	4.44	4.57	4.25	4.67	4.78	4.83	4.59

A16. Blood Pressure (mmHg)

	NO-L								PLA							
Subject	BP 1S	BP 1D	BP 2S	BP 2D	BP 3S	BP 3D	BP 4S	BP 4D	BP 1S	BP 1D	BP 2S	BP 2D	BP 3S	BP 3D	BP 4S	BP 4D
BLA	127	70	122	84	156	89	125	73	123	71	137	83	155	90	131	73
BLB	125	72	111	69	150	78	120	72	128	72	114	78	148	98	128	74
JRB	108	73	100	76	126	79	100	55	102	71	110	78	130	68	92	61
BMK	129	78	136	85	172	100	137	82	122	72	130	79	171	89	133	69
TLB	121	58	126	71	153	69	119	69	114	64	141	74	161	82	143	81
JMY	104	69	110	84	132	80	114	71	103	69	104	75	121	72	99	69
CWC	114	71	120	71	167	86	136	98	115	75	127	79	162	78	140	82
OKA	108	59	107	60	135	91	109	69	115	56	108	73	147	71	122	70
ERC	115	62	124	82	132	92	130	87	117	73	104	74	128	80	117	74
SYK	114	71	120	66	123	76	117	77	111	69	114	72	120	77	112	74
NLK	104	60	102	71	144	86	110	67	115	73	105	75	167	85	126	68
LFA	99	61	108	67	150	82	116	70	121	65	130	76	163	80	118	74
BTA	129	78	135	89	157	91	141	83	122	67	112	80	163	86	140	74
SMV	118	77	116	80	142	88	105	55	114	81	113	73	137	85	102	64

BP 1: Pre-resting, BP 2: Post-resting, BP 3: Exercise, BP 4: Recovery& S: systolic, D: diastolic

A17. Mean Arterial Pressure (mmHg)

	NO-L				PLA			
Subject	Pre-resting	Post-resting	Exercise	Recovery	Pre-resting	Post-resting	Exercise	Recovery
BLA	71	83	90	73	70	84	89	73
BLB	72	78	98	74	72	69	78	72
JRB	71	78	68	61	73	76	79	55
BMK	72	79	89	69	78	85	100	82
TLB	64	74	82	81	58	71	69	69
JMY	69	75	72	69	69	84	80	71
CWC	75	79	78	82	71	71	86	98
OKA	56	73	71	70	59	60	91	69
ERC	73	74	80	74	62	82	92	87
SYK	69	72	77	74	71	66	76	77
NLK	73	75	85	68	60	71	86	67
LFA	65	76	80	74	61	67	82	70
BTA	85	91	112	96	95	104	113	102
SMV	91	92	106	72	92	86	102	77

A18. Heart Rates (beat per minute)

	NO-L												
Subject	HR 1	HR 2	HR 3	HR 4	HR 5	HR 6	HR 7	HR 8	HR 9	HR 10	HR 11	HR 12	HR 13
BLA	57	65	160	157	168	173	116	101	101	92	95	93	86
BLB	67	63	168	159	161	166	101	91	85	86	86	83	72
JRB	79	75	122	126	121	137	106	92	87	84	87	88	83
BMK	61	59	142	154	164	164	121	98	96	95	94	92	90
TLB	56	59	150	150	155	158	111	100	99	103	94	87	87
JMY	85	61	139	147	144	153	116	109	106	101	100	102	85
CWC	63	62	147	146	156	161	101	82	84	90	88	83	88
OKA	59	59	138	118	137	128	67	74	71	65	63	69	69
ERC	57	62	133	122	172	178	121	101	97	97	88	96	92
SYK	58	58	162	164	170	172	121	100	93	89	87	84	81
NLK	54	56	162	166	171	175	121	115	102	100	102	104	99
LFA	51	53	127	126	132	136	84	81	74	72	75	72	70
BTA	76	77	143	153	156	158	124	109	107	110	105	105	101
SMV	63	59	154	154	164	170	105	85	87	83	80	84	82

	PLA												
Subject	HR 1	HR 2	HR 3	HR 4	HR 5	HR 6	HR 7	HR 8	HR 9	HR 10	HR 11	HR 12	HR 13
BLA	65	65	152	153	170	175	125	115	100	99	96	94	83
BLB	72	64	169	162	163	163	99	92	89	88	86	86	77
JRB	73	70	126	124	129	125	98	85	88	81	79	82	74
BMK	65	65	136	150	150	159	113	100	92	91	88	92	80
TLB	51	53	158	156	156	161	119	116	108	103	96	94	94
JMY	69	70	141	148	153	162	121	119	111	107	105	104	98
CWC	65	67	142	157	165	172	106	122	103	105	90	88	88
OKA	55	58	128	128	126	118	67	64	68	62	71	67	70
ERC	50	60	162	159	161	166	97	80	73	70	75	75	89
SYK	62	69	145	158	158	163	95	95	87	85	82	79	77
NLK	60	56	164	166	174	176	120	102	97	97	95	98	93
LFA	52	52	126	127	136	141	85	79	76	71	75	73	70
BTA	67	74	127	145	149	151	113	104	98	95	92	90	91
SMV	67	62	143	150	152	162	94	93	78	85	84	79	66

HR 1: Pre-resting, HR 2: Post-resting, HR 3 to HR 6: Exercise 1^{st} to 4^{th}, HR 7 to HR 13: Recovery of 4^{th} to 10^{th}

A19. Estimated Each Exercise Set Power (watts)

	NO-L				PLA			
Subject	1st set	2nd set	3rd set	4th set	1st set	2nd set	3rd set	4th set
BLA	302.8	229.5	205.5	196.6	309.3	245.3	221.8	196.6
BLB	332.9	247.3	220.5	217.5	354.3	236.5	214.8	199.9
JRB	304.5	256.5	239.7	246.5	309.7	255.4	254.2	235.6
BMK	327.5	224.6	182.1	162.5	341.7	215.3	157.6	162.8
TLB	344.2	210.3	151.4	129.2	357.1	210.7	163.4	147.3
JMY	313.8	178.3	154.1	167.5	318.2	189.2	162.8	160.3
CWC	355.1	229.2	199.8	197.5	343.4	241.5	196.2	188.7
OKA	291.9	229.9	200.7	185.9	323.3	228.7	185.5	167.6
ERC	298.3	212.0	190.2	170.3	252.4	179.5	160.5	158.1
SYK	426.8	249.2	211.2	193.1	371.7	284.3	208.4	212.8
NLK	380.9	263.1	233.0	209.8	381.9	272.5	226.1	217.8
LFA	351.1	236.0	215.7	214.8	352.6	244.1	229.9	215.4
BTA	408.7	319.5	245.7	224.4	404.3	332.8	236.1	232.8
SMV	340.2	237.3	203.3	188.9	335.0	221.4	186.5	177.8

Estimated workload = each set work done × 0.0226 × 2 × 60/32

Appendix B. Method Details

B1. VO_{2max} Test Protocol for Non-Athlete Active Subject

Stage	Watts (circle protocol chosen)			Record Time	RPE (6-20)	HR (bpm)
	Female	Male				
		Low<80kg	High≥80kg			
1(00:00-02:00)	100	100	100	01:50		
2(02:00-03:00)	115	115	120			
3(03:00-04:00)	130	130	140	03:50		
4(04:00-05:00)	145	145	160			
5(05:00-06:00)	160	160	180	05:50		
6(06:00-07:00)	175	175	200			
7(07:00-08:00)	190	190	220	07:50		
8(08:00-09:00)	200	205	240			
9(09:00-10:00)	210	220	260	09:50		
10(10:00-11:00)	220	235	280			
11(11:00-12:00)	230	250	300	11:50		
12(12:00-13:00)	240	265	320	12:50		
13(13:00-14:00)	250	280	340	13:50		
14(14:00-15:00)	260	295	360	14:50		
15(15:00-16:00)	270	310	380	15:50		
Cool down	Stop Time: ____:____	Max Watts: ___	HR_{max} (bpm): ____	VO_{2max} (ml/kg/min): _____	Min 5: HR _____bpm	

Subjects warmed up for 5min with cycling ergometer or on the floor before starting VO_{2max} test. Exact indication was given and urged subjects to keep the minimal 60RPM when they pointed out Borg scale "very hard". 0 workload was given right after they were all-out to cool down properly and recovery heart rates was continuously monitored during 5min after the test.

B2. Experimental Protocol Time Frame

Protocol Stage	Time Before &After Treatment (Min: sec)	Interval Time Between stages (Min: sec)	Measurements In order
Subject Check-in	- 20:00	5	
Baseline (Pre treatment)	- 15:00	10	MC, HR, BP, BD
Study product intake	- 5:00	35	
Baseline (Post treatment, pre exercise)	30:00	10	MC, HR, BP, BD
Resistance exercise 1st set	40:00 to 40:30	00:30	MC, HR
Resistance exercise 2nd set	41:00 to 41:30	00:30	MC, HR
Resistance exercise 3rd set	42:00 to 42:30	00:30	MC, HR
Resistance exercise 4th set	43:00 to 43:30	02:00	MC, HR, BD, BP
1st one maximal contraction	45:30	02:01	MC, HR
2nd one maximal contraction	47:31	02:01	MC, HR
3rd one maximal contraction	49:32	02:01	MC, HR
4th one maximal contraction	51:33	02:01	MC, HR
5th one maximal contraction	53:34	02:01	MC, HR
6th one maximal contraction	55:35	00:01	MC, HR
End of last one maximal contraction	57:36		BD, BP, HR

Blood Draw (BD), Blood Pressure (BP), Heart Rate (HR), Metabolic Cart (MC)

B3. Overall Procedure

	Pre-treatment				**Post-treatment**						
		Check-in		**Resting**		**Resting**		**Exercise**		**Recovery**	**Check-out**
Trial 1	1st Supplementation 2 days before 2nd Supplementation 1 day before	↑ BM FBG	↑ **BD 1** BP 1	↑ 5 min MG HR1	3rd Supplementation on the trial day **30min interval b/w resting**	↑ 5 min MG HR2	↑ **BD 2** BP 2	↑ 4 min MG HR3~6	↑ **BD 3** BP 3	↑ 13 min MG HR7~12	↑ **BD 4** BP 4 HR13
					↑ **1 week apart** ↓						
		Check-in		**Resting**		**Resting**		**Exercise**		**Recovery**	**Check-out**
Trial 2	1st Supplementation 2 days before 2nd Supplementation 1 day before	↑ BM FBG	↑ **BD 1** BP 1	↑ 5 min MG HR1	3rd Supplementation on the trial day **30min interval b/w resting**	↑ 5 min MG HR2	↑ **BD 2** BP 2	↑ 4 min MG HR3~6	↑ **BD 3** BP 3	↑ 13 min MG HR7~12	↑ **BD 4** BP 4 HR13

BM: body mass, FBG: fasting blood glucose, BD: blood drawing, BP: blood pressure, MG: metabolic gas, HR: heart rate

B4. Resting and Exercise Positions

1. Resting position

2. Exercise position

Isokinetic knee exercise: isokinetic knee exercise was designed to measure repeated peak torques and total work done during extensor/flexor muscle contractions at dominant leg only. The Biodex dynamometer system (Biodex, 900-760, USA) was set up to support concentric contractions only at 180°/sec of angular velocity. The range of motion for the knee extension/flexion was set up at 90°. Muscle force and power was determined for each contraction. Subjects performed maximal knee muscle contractions at 180°/sec angular speed for 4sets × 28 reps with 30 seconds of rest between sets. Following the isokinetic knee extension/flexion bout, the subjects performed one maximal isokinetic contraction every 2 min for 12 minutes.

B5. Making Solutions

How to make solutions for aliquot

- 200ml EDTA (pH 7.4)

  Preparation: 4.8g of ED2SS powder (24mgper ml), distilled water, pH meter, stirring magnet, and beaker

  1.150ml of distilled water + 4.8g of ED2SS into the beaker& stirring

  2. Adjusting pH with NaOH and HCl to the 7.4

  3. Adding distilled water up to 200ml with adjusting pH little

- 50ml Methanol

  : 99.9% methanol (F.W: 32.04) into the 50ml effendorf tube

- 200ml 10% PCA

  Preparation: 70% perchloric acid liquid, distilled water, stirring magnet, and beaker

  1. 150ml of distilled water + 28.57ml of 70 % perchloric acid liquid into the beaker& stirring

  2. Adding distilled water up to 200ml

How to make solutions and reagents for lactate sample assay

- Reagent cocktail for each tube

  Preparation: 1ml glycine – hydrazine buffer, 0.83mg of NAD, 5µl of LDH, stirring magnet, and 50ml effendorf tube or beaker

  1. Calculating the #of tubes considering 2~3ml extra

  2. Glycine –hydrazine buffer, NAD, LDH into the effendorf tube or beaker and mixing

- 1000ml glycine – hydrazine buffer (pH9.8)

  Preparation: 0.33M glycine 25.02g, 0.27M hydrazine 23.98ml, distilled water, pH meter, stirring magnet, and beaker

  1. 900ml of distilled water + 22.02g of glycine powder + 23.98ml of 0.27M hydrazine

  2. Adjusting pH with NaOH and HCl to the 9.8

  3. Adding distilled water up to 1000ml with adjusting pH little

B6. Tube Labeling

Label was marked on top and side of empty 1.5 ml tube as following method.

Abbreviation

NO-L: NO-L supplementation study

NOx: Plasma nitrate and nitrite sample

Lac: Whole blood lactate sample

Glu: Serum glucose sample

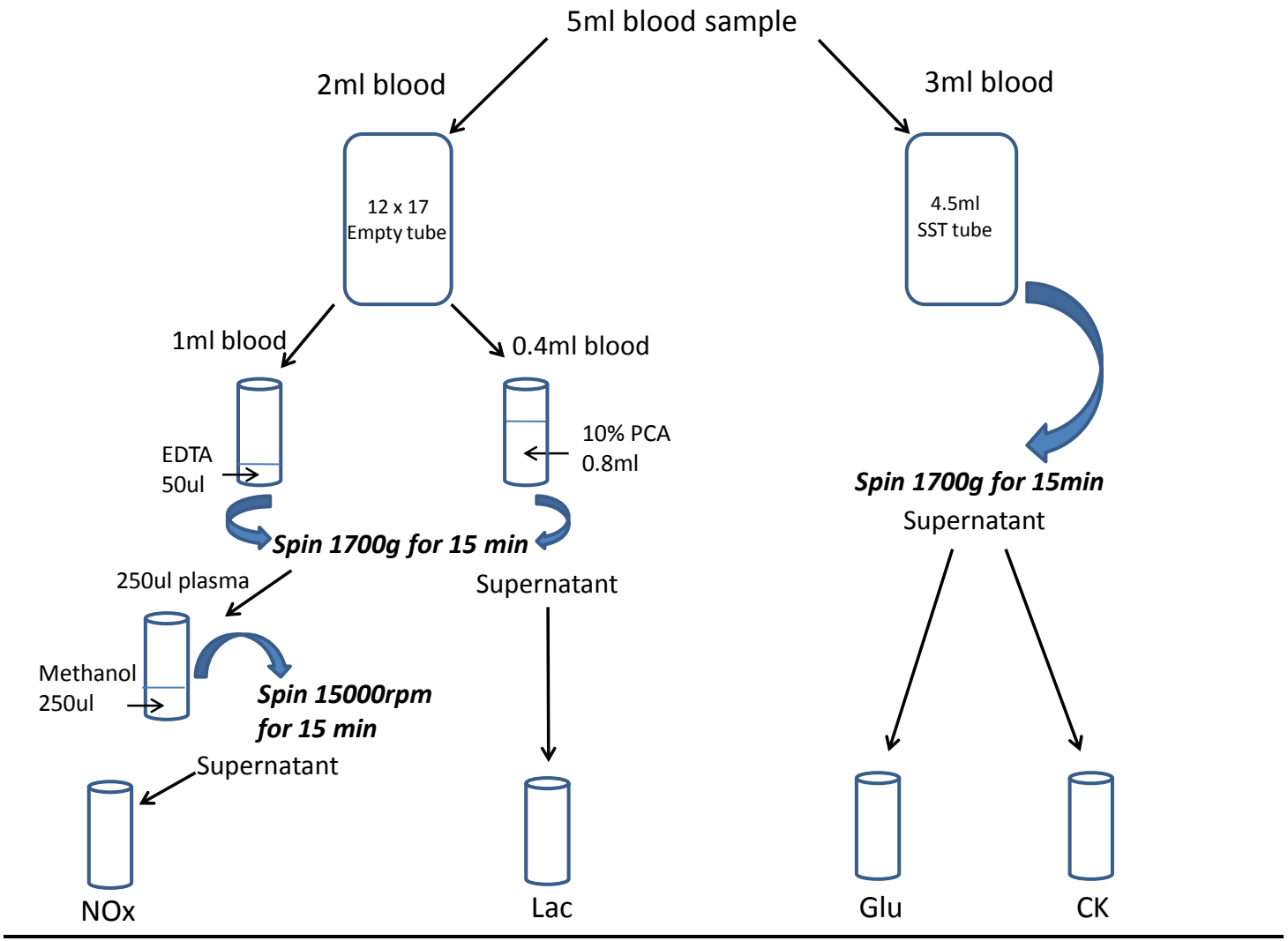
T #: Trial order

BD #: Blood sampling order

For example, if the subject's name is Kyoungrae Kim and it is 1st trail 1 for blood samples,

NO-L NOx KRK-T1 BD1	→	NO-L NOx KRK-T1 BD2	→	NO-L NOx KRK-T1 BD3	→	NO-L NOx KRK-T1 BD4
NO-L Lac KRK-T1 BD1	→	NO-L Lac KRK-T1 BD2	→	NO-L Lac KRK-T1 BD3	→	NO-L Lac KRK-T1 BD4
NO-L Glu KRK-T1 BD1	→	NO-L Glu KRK-T1 BD2	→	NO-L Glu KRK-T1 BD3	→	NO-L Glu KRK-T1 BD4

B7. Blood Handling



B7.1. Blood Handling Detail

BD1

EDTA BD1 & PCA BD1 15min At 1700 RCF **SST BD1** 45min at RT 45min at 0◦C	→	250µl of plasma into the **Met BD1** 15min 15000 RPM Supernatant of PCA into the sample tube **(LacBD1)**	→	Supernatant of Met BD1 into the sample tube **(NOx BD1)**	→	Every sample tubes were kept in the 0˚C ice box until move into the -80˚C refrigerator **EDTA BD2~4 & PCA BD2~4** Kept in the 0˚C ice box until s of BD4 is finished

BD2 to BD4

EDTA BD2~4 & PCA BD2~4 15min At 1700 RCF **SST BD1~4** 15min at 1700 RCF	→	250µl of plasma into the **Met BD2~4** 15min 15000 RPM Supernatant of PCA into the sample tube **(LacBD2~4)** Supernatant of SST Into the sample tube **(Glu& CK BD2~4)**	→	Supernatant of Met BD2~4 into the sample tube **(NOx BD2~4)**	→	Every sample tubes were move into the -80˚C refrigerator immediately after every sampling was finished

B8. Determining Sample Size and Power

Method1. G power 3.1.7

Reference	Dependent Variables Test Family	Power	P value	Effect Size	Tailed	Minimum Sample Size Actual Power
Fulford et al., 2013	PCr cost Paired t-test	.80	.05	.580	One-tailed	20 .80
Larsen et al., 2011	PO ratio Paired t-test	.80	.05	1.07	Two-tailed	9 .802

Reference

Fulford J, Winyard PG, Vanhatalo A, Bailey SJ, Blackwell JR, Jones AM.

Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Pflugers Arch*. 2013. 465(4): 517-28.

Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg

E. Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab*.2011. 13(2): 149-59.

Method2. Normal range of sample size

Minimum: 7 (Bailey et al., 2010)

Maximum: 15(Masschleinet al., 2012)

Averaging: usually 9-12 males participate

My decision for proper sample size and power of NO-L study was 12 - 14 males.

Appendix C. Procedure Details

C1. Randomization

Subject	T1 treatment	T2 treatment
BLA	PLA	NO-L
BLB	PLA	NO-L
JRB	PLA	NO-L
BMK	NO-L	PLA
TLB	PLA	NO-L
JMY	PLA	NO-L
CWC	NO-L	PLA
OKA	PLA	NO
ERC	NO-L	PLA
SYK	NO-L	PLA
NLK	NO-L	PLA
LFA	NO-L	PLA
BTA	NO-L	PLA
SMV	PLA	NO-L
Randomization	**NO-L=7 vs. PLA=7**	**NO-L=7 vs. PLA=7**

C2. Participant Visit Schedules

Mon	Tue	Wed	Thurs	Fri	Sat
5/12 10:20 BMK – V1	5/13 08:20 TLB – V1	5/14	5/15 08:00 CWC – V1 10:30 OKA – V1	5/16 08:30 ERC – V1	5/17
5/19	5/20 17:30 SYK – V1	5/21	5/22 11:00 NLK – V1	5/23	5/24
5/26	5/27 08:00 TLB- FAM	5/28 08:00 OKA - FAM	5/29 08:00 CWC – FAM	5/30 08:00 ERC - FAM	5/31
6/2	6/3 08:00 TLB- T1	6/4 08:00 OKA – T1	6/5 08:00 CWC – T1	6/6 08:00 ERC – T1	6/7
6/9 07:00 SYK – FAM 15:00 LFA – V1	6/10 08:00 TLB- T2	6/11 08:00 OKA – T2 10:30 BLB – FAM	6/12 08:00 CWC – T2	6/13 08:00 ERC – T2 10:30 NLK – FAM	6/14
6/16 07:00 SYK – T1	6/17 13:30 JRB – V1	6/18 08:00 LFA – FAM 10:30 BLB – T1	6/19	6/20 08:00 BLA – FAM 10:30 NLK – T1	6/21 10:00 BMK – FAM
6/23 07:00 SYK – T2 11:00 JRB – FAM	6/24 16:40 JMY – V1	6/25 08:00 LFA – T1 10:30 BLB – T2	6/26	6/27 08:00 BLA – T1 10:30 NLK – T2	6/28 12:30 JMY – FAM
6/30 11:00 JRB – T1 14:00 BMK – T1	7/1	7/2 08:00 LFA – T2	7/3 10:30 JMY – T1	7/4	7/5
7/7 11:00 JRB – T2 14:00 BMK – T2	7/8 9/2 13:30 BTA –V1	7/9	7/10 10:30 JMY – T2	7/11 08:00 BLA – T2 9/5 BTA - FAM	7/12
9/8	9/9	9/10	9/11 15:30 SMV – V1	9/12 11:30 SMV – FAM	9/13
9/15	9/16	9/17	9/18	9/19 09:00 BTA – T1 11:30 SMV – T1	9/20
9/22	9/23	9/24	9/25	9/26 09:00 BTA – T2 11:30 SMV – T2	9/27

C3. Supplies

Supplements

FAM	Trial 1	Trial 2
. 2 lozenges for 2 days before the trial 1	. 1 lozenge for the trial 1	. 1 lozenge for the trail 2
. Instruction how to intake the tablet	. 2 lozenges for 2 days before the trial 2	

Reminder Email and texting (Every day checking)

: Reminder email 2 days before the Screening
: Text message 1 day before the Screening
: Reminder email 3 days before the FAM, T1, and T2
: Text message 2 days before the FAM, T1, and T2

Blood sampling

: Catheter, saline pack, Tegaderm^{TM} film, connecter line, luer access split septum, 20G1 needle, gauze sponges, alcohol prep pad, diaper, gloves, hand sanitizer, small timer, tourniquet, tapes
: Blood glucose monitor

Aliquot

: 50 µ EDTA into the ml tubes, 250 µl Methanol into the ml tube, 800 µl 10% PCA into the ml
: Everyday sample tubes labeling and double check with Bo
: Icebox with ice, 3 empty plastic cups for blood stuffs
: Water filled beaker, (½ water + ½ bleach) filled beaker
: 1 ml, 0.4ml, and 0.25ml pipettes

Metabolic gas collecting

: Blood pressure monitor, heart rate monitor, two master timers, and hose

C4. How to take the Study Supplements

1. You will be given two lozenges in a sealed plastic bag each week to take home with you. Keep the bag sealed and airtight.
2. The lozenges (tablets) are designed to dissolve in your mouth. Just suck on the lozenge until it is completely dissolved. Do not chew it.
3. Take one lozenge when you first wake up two days before your experimental trial and the second lozenge one day before your experimental trial. For example, if you are being tested on Tuesday, you would take one lozenge on Sunday and one on Monday.
4. Do not brush teeth within 30 minutes of taking the lozenge. It is ok to eat after taking the lozenge.
5. Do not use mouth wash at anytime during the two weeks you are being tested (starting when we give you your first two lozenges)
6. When you open the plastic bag to remove the first lozenge, make sure you get as much air out of the bag before resealing it.

C5. Quick Manual of FAM, T1, and T2

Time	**Procedures**		
- 20 min	□ Check-in Time**____: ____ AM / PM** □ Check no caffeine for 12h and no strenuous exercise and no alcohol for 24h with enough water during fasting □ Record time of last meal **____: ____ AM / PM** □ Check **no mouthwash in the morning,** Brushing teeth (No, Yes)____: ____ AM / PM □ Have subject use restroom at 9thfloor □ Check subject's body mass _____**lb.**_____**Kg** at 8th floor □ Ask subject to drink water (800ml -> _____ ml) and explain no water will be served till end of the test □ Check blood glucose _____**mg/dl**□ Verifying heart rates monitor (Tech 1) □ Catheter insertion→ □ **BD1**→ □ Flush each 6 – 7 min by using small timer (Lead), 2min break□ **BP1**____ /____ mmHg (Tech 1)		
Pre-Treatment Resting Baseline			
-10min	□ Have subject sit in Biodex □ 5min baseline gas collection, HR_____ bpm at 3 min (Tech 1) □ Generate report summary: Name – FAM, T1, or T2 – Pre treatment resting– Beet Elite TM		
- 4 min	□ Have subject move to the chair□ Have subject **take study product** and finish it		
Post-Treatment Resting Baseline			
0 min	□**Start** master timers<u>00:00,</u> Pre-set position and ROM 0 – 90 degree (Max GET)		
27 min	□ Have subject sit in Biodex and prepare for gas analysis (Tech 1)		
30 min	□ Start metabolic cart, HR_____ bpm at 3 min (Tech 1)		
35 min	□ **Pause** metabolic cart at 05:00 min(Tech 1) □ **BD 2 →**□ **BP 2** ____ /____ mmHg (Tech 1)		
~ 40 min	□ Set Biodex position, waist belt, chest belt, leg belt, ankle adaptor		
Post-Treatment Exercise / Recovery			
At 05:10 Of MC	□ **Resume** metabolic cart at 05:00 of MC □ Start 1st set at 05:10 until 4th set of Biodex exercise □**Record master timer ___: ___** (Tech 1)	MC timer 1st end ___: ___ (Tech 1) MC timer 2nd end ___: ___ (Tech 1) MC timer 3rd end ___: ___ (Tech 1) MC timer 4th end ___: ___ (Tech 1)	HR 1_____bpm HR 2_____bpm HR 3_____bpm HR 4_____bpm
	□ **BD 3→** □ **BP 3**____ /____ mmHg (Tech 1), Recovery HR measurement: 15 sec before every single kick		
	□ Start 5th set and keep to the end of Biodex exercise □ MC timer 5th start ___: ___ (Tech 1) □ MC timer 10th end ___: ___ (Tech 1)	HR 5_____bpmHR 8_____bpm HR 6_____bpm HR 9_____bpm HR 7_____bpmHR 10_____ bpm	

At 22:00 Of MC	□ **BD 4** □ Stop metabolic cart **at 22:00** (Tech 1) □ **Record master timer ___: ___** (Tech 1) □Remove head gear set →have subject sit in the chair →□HR 11_____ bpm, □**BP 4** ____ /____ mmHg (Tech 1) □ Generate report summary: Name – FAM, T1, or T2 – Post treatment – Beet Elite TM
	□ Give subject water □ Give subject 2 lozenges of placebo or treatment for next visit □ Explain the administration method and precautions for 2 days prior to their appointment □ Give subject beverage and 1 or 2 energy bar □ Print out food logs and exercise logs at 818
	□ Check-out Time**____: ____ AM / PM** □ Tidy up 902, clean everything, place everything in its place □Prepare stuffs for next experiment, and confirm with Trial protocol

Comments: BD5 time:

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